fraction (>2.36 mm) would take between 47 s and 3 min to settle 5 and 20 m, respectively. Assuming that the smallest fraction (<53 μm) would settle, it would take coal particles of this size fraction between 52 min and 21 h to settle the same depths. However, both of these calculations assume that the water column lacks any vertical or horizontal currents. Such conditions are rare at Roberts Bank, and would only occur at slack tide on an extremely calm day. The action of any currents in the water column would have drastic repercussions on the settling velocities of the coal particles, especially for the smaller size fractions. ` On Roberts Bank, normal maximum tidal currents alone can reach 0.051–0.76 m/s near Pod #1 (Canadian Hydrographic Service). In such currents the coal particles larger than 2.36 mm could travel laterally up to approximately 60 m to settle 5 m through the water column, and travel 230 m to settle 20 m. In the same currents, the smallest size fraction could travel between 4 and 96 km laterally to settle to the same depths (although it is highly unlikely that maximum tidal currents could be sustained for the 21 h necessary for 96 km of dispersal). Upwelling currents and turbulence would also contribute to the residence time of the coal particles in the water column. Furthermore, these calculations assume that the particles would settle in the first place, though the waves and currents would undoubtedly agitate the coal particles to a degree and initiate settling.

The hydrophobicity of the coal particles would result in particles staying afloat longer than assumed in the above calculations. During the sampling a thin layer of small coal particles floating on calm water approximately 200 m east of Pod #2 was observed. This film of fine coal particles was observed when there was no coal loading activities in progress, and no ship was docked.

The distribution of coal around Westshore Terminals is in agreement with the data from the analysis of the coal settling properties. The sediments that contain the highest coal concentrations are in the coarser size fractions in close proximity to the coal loading facility. The experiments studying the settling velocities of the coal particles indicate that the larger coal particles (with settling velocities of up to 10.54 cm/s) settle out within the first few hundred metres of the terminal (depending on the currents). The degree of coal oxidation would dictate which coal size fraction would readily settle, with the increasingly oxidized particles tending to settle due to their decreased hydrophobicity. The smaller particles (as well as those oxidized to a lesser degree) would float longer and take longer to settle (with minimum velocities of 0.16 cm/s) through the water column, resulting in an increased dispersal of the coal, and coincident decrease in the sediment coal content. These low concentrations would be difficult to detect using the hydrolysis method of this study.

Benthic flora and fauna, which are most susceptible to coal dust coverage and possible anoxic conditions that might arise due to the oxidation of the coal, would likely only be affected on sediments within very close proximity (0–300 m) to the coal loading terminals at pods #1 and 2. Creatures dwelling further away would unlikely experience coal concentrations sufficient to blanket the bottom (thereby decreasing insulation) and give rise to anoxic conditions in the upper sediments. Furthermore, in all of the sediments sampled in this study, the hydrolysable organic matter content of the sediments ranged between one-and-a-half to 20 times the coal content (NHS) of the sediments. If anoxic conditions were to arise, they would likely be the result of the natural organic detritus rather than the coal content. Inspection of the sediments around Westshore Terminals failed to reveal any evidence of anoxic conditions in the upper sediments. If anoxic conditions did prevail, the sediments (at a variable depth below the sediment–water interface, depending on the degree of oxidation) would be expected to have a dark (black) coloration and pungent aroma. Such characteristics would reflect a reducing environment in which bacterial degradation of the organic matter (as well as the activities of detritivores) was inhibited by a lack of oxygen.

The benthic creatures dwelling in the sediments adjacent to the coal terminal would more likely be adversely affected by the alteration of their habitat through changes in the physical nature of the substrate such as size, weight, particle shape, porosity, permeability, and stability of the sediments (Pearce and McBride, 1977) due to the dredging operations in the area.

Though this report does not directly address the amount of suspended solid levels (i.e. coal) in the waters around Westshore Terminals, Shelton (1971) documented the effects of dumping annually 6.2 million metric tonnes of fine coal, fly ash and other colliery wastes off the north coast of England. Investigations demonstrated that the growth of periphytic (attached) algae was inhibited by the reduction of light penetration from increased levels of suspended solid load, adversely affecting the fauna associated with the attached algae. While the volume of coal dust settling on Roberts Bank is undoubtedly much less than that documented by Shelton (1971), the study does indicate the effects of suspended coal levels may have on marine flora and fauna.

Acknowledgements

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E. CALVIN

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MINIMIZING GROUNDWATER CONSUMPTION FOR REQUIRED FUGITIVE DUST CONTROL PROGRAMS

George D. Emmitt
Executive Vice President/Senior Scientist
Simpson Weather Associates, Inc.
Charlottesville, Virginia

ABSTRACT

Control of fugitive dust emissions from coal ground storage facilities, in most cases, involves the use of water, applied with fixed and/or mobile water spray cannons. Based upon experience at Virginia coal transshipment facilities, the Department of Environmental Quality (DEQ) has issued operating permits that require the use of ~ 8 gallons of water per throughput ton of coal. A facility, with throughput of 20-40 million tons puts a rather large demand on municipal water supplies or local aquifers. In either case, there are incentives to minimize the need for such water. Recycling of dust control water and the capture/storage of rainfall are two options available to reduce the demand on potable water supplies.

Simpson Weather Associates (SWA) has developed and applied a full Water Budget Model (WBM) for designing and operating a fugitive dust control system at a coal ground storage facility. The model uses 30 years of hourly weather data to simulate a model facility's impact on fugitive dust management, storm water management, water treatment, and water discharge. The model yields optimum settling pond size and water retention schemes to

1) minimize groundwater demand;
2) minimize discharge to streams during a 25+ year storm;
3) meet dust control permit requirements during extended droughts; and
4) minimize suspension of operations due to insufficient water supply or local flooding.

INTRODUCTION

A common strategy for suppressing fugitive dust emissions from an industrial complex is to use water sprays delivered by tank trucks, in situ spray bars or elevated water cannons. In the case of the ground storage of coal, the water required to maintain dust control can be significant, on the order of several 100 million gallons of water per year. Both existing and proposed ground storage facilities for coal in Virginia are required by the DEQ to employ water (plus surfactants in some cases) to assure compliance with air quality standards for particulates. The quantities of water needed to meet the permit requirements can represent a significant demand on potable water, whether it is supplied via municipality water mains or from dedicated wells.

In addition to reducing the annual consumption of potable water, facility operators are concerned with designing water holding/treatment ponds that will be adequate for dry period (low rainfall) operations as well as sufficient for the retention of major storm water runoffs.. A Water Budget Model (WBM) has been developed to provide the basis for sizing the water delivery, retention, treatment and discharge components of the dust control/storm water runoff system at a large coal ground storage facility.

THE WATER BUDGET MODEL

A general flow diagram of the Water Budget Model is illustrated in Figure 1. The primary assumptions made by the model are that water is needed for:

1) dust control during coal handling, in particular, at the rotary dumper, stacker/reclaimer and loadout silos;
2) dust control for piles;
3) the cleaning of storage pads, vehicles and roadways;

4) fire control; and
5) incidental human consumption, cleaning, etc.

It is further taken that:

1) saline water is not acceptable;
2) municipal or other public water is not available;
3) rainwater will be captured and stored;
4) a portion of the water used in dust control will be recycled; and
5) ground water will be the source of "make-up" water.

The following discussion of the WBM is based upon a hypothetical design of a facility with a ground storage capacity of 4 million tons, an average working storage of 2.5 million tons, and an annual throughput of 40 million tons.

The WBM was run using three different weather scenarios - a typical year, a design storm and a design drought. The "typical year" data was obtained from a meteorological tower located in Newport News, VA. The criteria for spraying the coal piles was taken to be the K-system required by the Virginia DEQ and described in Emmitt et al. (1996).

A second simulation was done using a design storm of 7.6 inches in 24-hours based upon climatological data from the official NOAA National Weather Service (NWS) station at Norfolk International Airport. This design storm has a return interval of 25 years.

A third water budget was run for a 90-day drought during the months of May, June and July. While there is no official engineering "design drought" for a coal handling facility, it is critical to design the water supply system to handle an extended dry period.

Water Demands

The primary demands for water include the spraying of the coal during its unloading and loading (inline dust control), the spraying of the piles of coal using the K-system (Emmitt et al., 1996), the wetting of roadways and the cleaning of equipment.

Inline Dust Control

It was assumed that water was used in the rotary dumpers to form a curtain spray to contain fugitive emissions during dumping. Based upon experience at Virginia terminals, a nominal water requirement is .75 gal/ton of coal handled. The curtain sprays are operated in a fashion to optimize capture and reuse of the spray water. It is estimated that at least 33% of the spray water can be captured and returned to the water supply pond.

In recognition that some of the incoming coal will require additional water (plus surfactant) to control emissions during stackout, inline sprays (.75 gal/ton) are provided at two more locations. In reclaiming the coal, additional wetting capability (.75 gal/ton) is provided at three selected transfer points.

Based upon a throughput of 40 million tons per year, the reprocessing* of 4 million tons per year and assuming the estimated annual maximum water requirement, the inline dust control, (INLINE) was computed to be 195 million gallons per year (MGY) (assuming all coal is treated with water at all six spray points).

The actual amount of water added to any given coal shipment may vary, depending upon several factors. First, real-time dust monitors at the dumper and other transfer points serve to identify coals that need water for dust control. Second, the amount of water needed to achieve a targeted level of dust control may depend upon the use and effectiveness of a wetting agent (surfactant). In the absence of sufficient data, a very conservative estimate was made: on the average only 50% of the inline spray capacity would used on the total coal processed.

A further reduction of the "inline" demand is realized when weather conditions are incorporated into the dust control system. Based upon weather data taken from Norfolk, VA, there are approximately 100-120 days per year with rainfalls greater than .10 inch. According to hourly data, it rains 6 out of 24 hours on days with rain and thus no spray water would be needed during that time.

Combining these reduction factors the expected inline water requirements for a typical year was calculated by the simulation model to be 71 MGY.

Coal Pile Dust Control

Control of fugitive dust emissions from the coal piles represents the largest demand for water. Sizing the water supply/delivery system depends primarily upon the estimated water requirements for fugitive dust control during extended dry periods.

In estimating the average annual water demand for dust control from coal piles we have made several assumptions:

- the objective of the pile spray system is to deliver an average of .02" of water in one spray cycle to the surface area of each pile and the surrounding pad area. The value of .02" is based upon amounts found acceptable by the DEQ for coal storage facilities in the Norfolk area,

- except for very extreme (crisis) cases each pile will be sprayed, at most, once per hour, and

- a minimum of 4 spray cycles will be used each day except when it is raining, the temperature is below freezing, or it is foggy.

While we recognize that it may be possible to reduce the water demand by discriminating between coals of different dust potential or between used and unused portions of the storage pad, a maximum treatment is computed and used as a "reference". The amount of water required for a reference spray cycle (assuming no overlapping in coverage) is 84,025 gals/cycle.

To estimate the spray water required during a typical year we have considered the following factors:

- number of hours in a year during which its is raining, below freezing, foggy or snow covered; and

- the number of cycles that would be required under a current DEQ permit. Thirty year's worth of hourly meteorological data obtained locally are used.

To determine the number of water spray cycles that are required for dust control, one cannot use average climate statistics. Instead, hourly weather data must be used to simulate the sequence of demand for dust control. Based upon 30 years of recorded hourly data, the K-system operations were simulated and the average water required to treat the piles was estimated to be 160 MGY.

Road Sprays

The pile spraying system was assumed to cover the primary yard traffic routes and thus no additional water was needed for road surfaces. It was further assumed that any frequently used road not wetted by the coal pile spray system would be paved and cleaned periodically with a spray truck.

Wash Water

A value of 6% (based upon experience) of pile spray water required was used to estimate the amount of water needed per day to clean roadways, vehicles, and other equipment. It was further assumed that with the exception of the roadways, this cleaning would take place where the water would be recaptured for recycling. A recycle factor of 75% was assumed as a best guess.

Water Supplies

Groundwater should not be the primary source for meeting the demands of a coal storage facility. The annual average and daily maximum amounts of required ground water are the net results of a water budget study and are therefore discussed in Section 3. It is advantageous to find alternative water supplies and ways of increasing the efficiency of water usage.

<u>Rainfall</u>

Rainfall represents a significant source of water if properly captured and stored. Not only can rainfall be stored but it also negates the need for other water usage during and immediately after rain events.

Our estimates of rainwater available for capture are based upon the following:

48.5" (4.0') of rain per year

capture areas
- pad - 6.8×10^6 ft^2
- inbound loop (includes pad) - 19.3×10^6 ft^2
- outbound loop - $10.3 \times 10^6 \times ft^2$

runoff factor (% of incident water)
- bare ground - 25%
- pad - 95%
- coal piles - 73%

The total amount of rain water runoff potential (gals):

loop area (w/o pad)	171.3 MGY
pad (30% open)	57.5 MGY
coal piles	103.0 MGY
Total	331.8 MGY

While the amount of rain water potentially available for dust control exceeds the estimated water demands, not all of that water survives evaporation or can be stored. In our simulation we have made the conservative assumption that <u>only</u> if it rains more than 0.25 inches within a 24-hour period will there be any runoff from the pad and piles that is actually available for capture. In other words, 0.25 inches of rain is lost to pooling on tops of piles, inhibited flow in drainage ditches and evaporation.

The model facility has perimeter ditches. These ditches carry water from the non-pad areas into the storage ponds. However, only under very heavy rain conditions should there be significant return from those ditches. The amount of water from these ditches that can actually be captured for use in the dust control system will depend upon the antecedent conditions of the soil and the pond levels at the time of the rainfall event. This potential water supply is not incorporated into the WBM at this time.

<u>Coal Pile Flow Through</u>

This is the most difficult term in the water budget to assess. It also has the potential of being a significant source of water. By "coal water yield" we include any water that exits the pile from the bottom. This water could be coal moisture that was imported with the coal or it could be some of the rain or spray water that infiltrated and flowed through the pile.

Using soil analogies (sand/silt) we can only make a very rough estimate of what this term might be. If the coal looses 1% weight due to gravitational drainage, then there is ~100 MGY available for retention. Based upon laboratory experiments, we estimate that the infiltration water (from rain) is released by the pile within 24 hours.

There is significant uncertainty in these estimates. There is the likelihood that much of the drainage would occur during non-rainy periods and would therefore evaporate before getting to the retention pond. We have assumed that 75% of the leachate will evaporate before being captured. The total flow through is reported primarily for water chemistry computations.

Recycled Water

Not all of the water sprayed on or toward the coal is absorbed. This is particularly true for the in-line dust control at the rotary dumper and loadout silos. Some of this water can be captured and recycled. The exact amount depends on the design of the dust control system. For the model facility we have assumed that 33% of the rotary dumper water and 75% of the water used to clean pads and vehicles can be recycled. All other inline spraying should be absorbed by the coal and effect the desired dust control.

WATER BUDGET RESULTS

All of the factors and budget components discussed in the preceding sections have been incorporated into the Water Budget Model. Thirty individual years of operation were simulated producing time series such as the one for 1974 shown in Figure 2. The model was also run for 2 design situations, the results for which follow.

Typical Year

A plot of the daily water demand during a 'typical year' for dust control and the groundwater requirements to maintain pond levels is provided in Figure 3. A summary of the water budget for this typical year follows:

Kilogallons of water associated with:	
Inline dust control	71099
Pile dust control (2668 cycles)	26491
Wash water	13589
Total water demand	311179
Captured rain water	113108
Recycled control water	14885
Pile flow through water	121440
Evaporation	99962
Total surface supply	149470
Groundwater required	161581
Total water supply	311051
Discharge required	819
Total rainfall (inches)	48.47

From this simulation we see that with a facility designed to use storm runoff, pile drainage and recycling of dust suppression water can meet nearly 48% of its water needs without use of fresh water from the ground supply. Given the limited capacity for storage of storm runoff, approximately ~1 MGY must be discharged. However, even this amount of "lost" water can be reduced by using the storage pads (with a berm) as a surge pond during heavy rains.

A Year With a 90-day Drought

In this simulation (Figure 4) we have used the same data used in the typical year case but have zeroed the rain for 90 consecutive days and have kept the minimum number of pile spray cycles per day at 4.

Kilogallons of water associated with:

Inline dust control	72205
Pile dust control (2813 cycles)	238807
Wash water	14328
Total water demand	325340
Captured rain water	77804
Recycled control water	15512
Pile flow through water	115088
Evaporation	95119
Total surface supply	113205
Groundwater required	211187
Total water supply	324392
Discharge required	0
Total rainfall (inches)	33.72

It is clear from the table above that the demand for water only increased 14 MG for the year (section 4.1) but the groundwater requirement increased by nearly 50 MG, due to the extended absence of rainfall.

SUMMARY

A water budget model has been developed and applied to a 'model' coal ground storage facility to provide the basis for designing the dust control water supply system and predicting the discharge of treated water. A properly designed facility can provide nearly 50% of its water requirements through the capture of rainwater, and the recycling of dust control water, combined with adequate sizing of retention and treatment ponds.

ACKNOWLEDGMENT

A portion of the development of the WBM was made possible through support from the Norfolk Southern Corporation.

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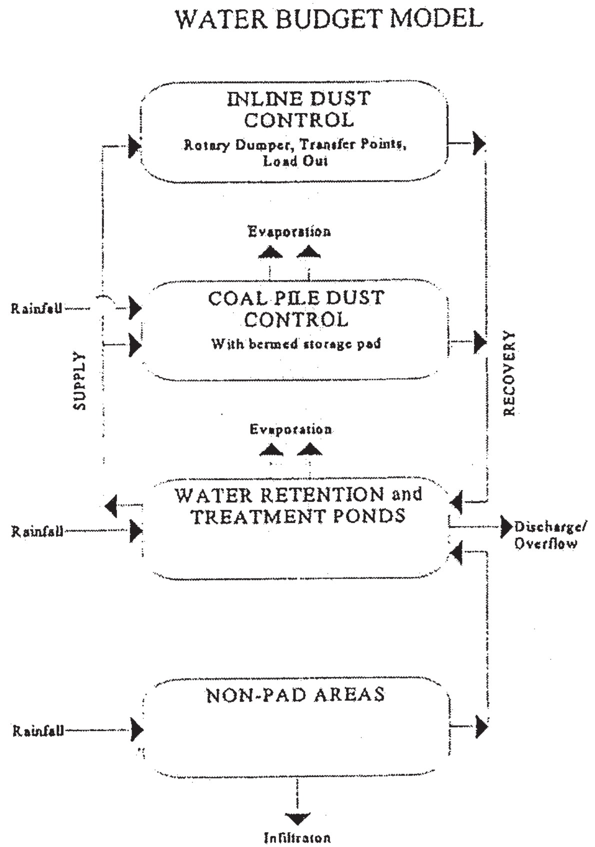


Figure 1. Flow Diagram for Water Model Budget

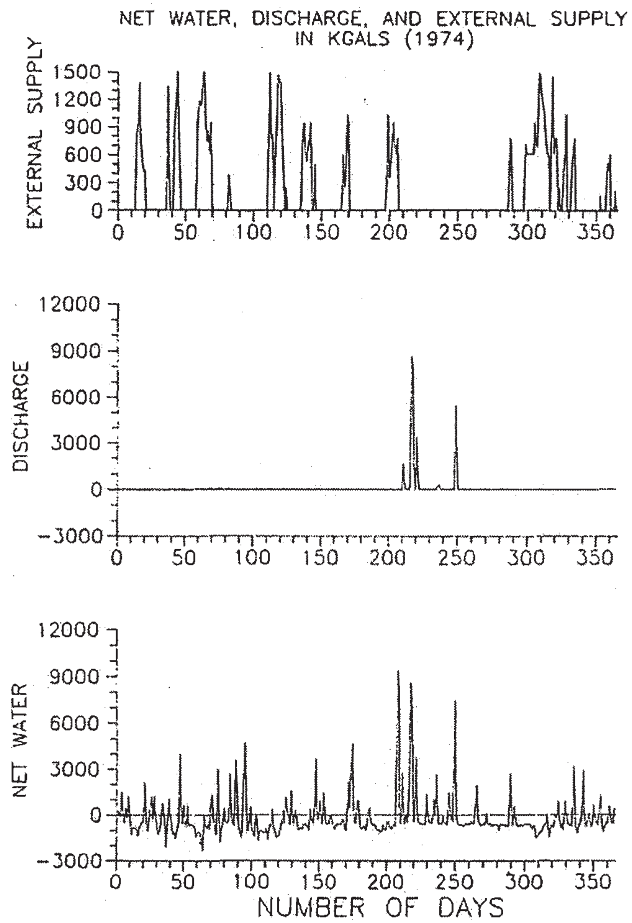


Figure 2. Example of One Year's WBM Simulation of the Model Coal Handling Facility

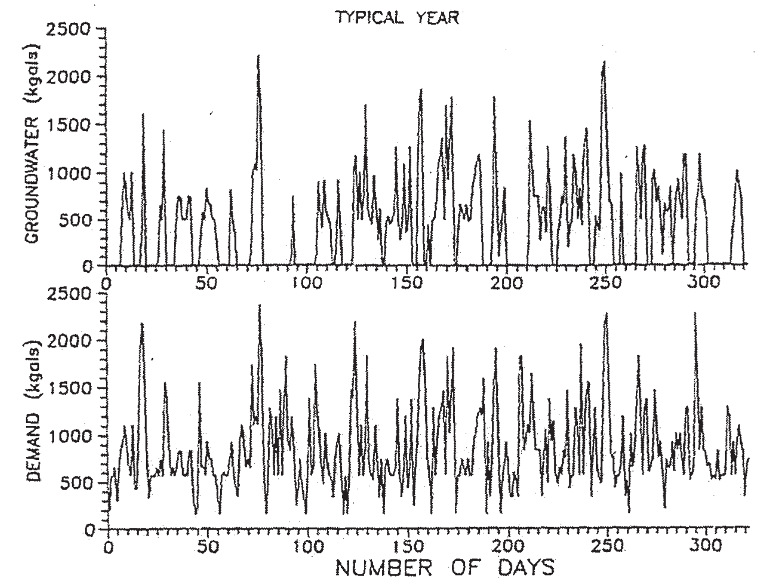


Figure 3. DEMAND (Dust Control) and GROUNDWATER (Municipal Supply)

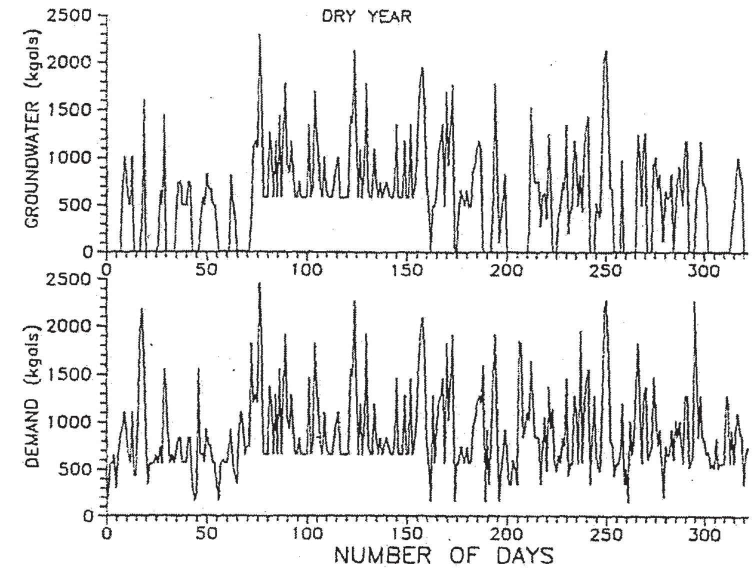
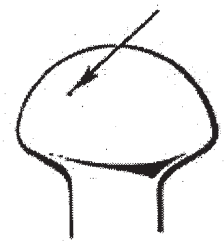


Figure 4. Same as Figure 3 Except a 90-Day Dry Period is Included

The Role of Chemicals in Controlling Coal Dust Emissions

By
Christopher F. Blazek, VP Marketing, Benetech Inc.
Presented at the American Coal Council
PRB Coal Use: Risk Management Strategies & Tactics Course
June 25, 26, 2003, Hyatt Regency Dearborn, Michigan

Defining the Dust Issue



A Micron-Size Dust Particle on a Pin Head

Dust consists of solid particles carried by air currents. Coal dust originates at impact points (including crushing and grinding), from previous accumulations, or from weathering. A wide range of particle sizes can be produced during a dust generating process. Larger particles settle more quickly than smaller particles, and the smallest particles can remain in the air indefinitely. Dust is typically measured in micrometers (commonly known as microns). Coal dust can range is size from over 100 µm to less than 2 µm. As a comparison, red blood cells are typical 8 µm and human hair ranges from 50-75 µm in size

In coal processing operations, dust is generated-

- When coal is broken by impact, abrasion, crushing, grinding, etc.
- Through release of previously generated dust during operations such as loading, dumping, and transferring
- Through recirculation of previously generated dust by wind or by the movement of workers and machinery

The amount of dust emitted by these activities depends on the physical characteristics of the material and the way in which the material is handled.

Fibrogenic dust, such as free crystalline silica or asbestos, is biologically toxic and, if retained in the lungs, can form scar tissue and impair the lungs' ability to function properly. PRB coal dust exceeds this 1% silica content as is regulated by OSHA to a level not to exceed 2.0 µm/m^3 of air volume. Furthermore, excessive concentrations of dust in the workplace may reduce visibility, may cause unpleasant deposits in eyes and nasal passages, and may cause injury to the skin or mucous membranes by chemical or mechanical action. From an occupational health view point, dust is classified by size into three primary categories:

- Respirable dust
- Inhalable dust
- Total dust

Respirable dust refers to those dust particles that are small enough to penetrate the nose, upper respiratory system, and travel deep into the lungs. Generally, the body has little ability to remove this respirable dust from the lungs. IMHA defines respirable dust as the fraction of airborne dust that passes a through a sieve, with 100% passing through 10 µm. The EPA describes inhalable dust as that size fraction of dust which

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enters the body, but is trapped in the nose, throat, and upper respiratory tract. The diameter of this dust is about 10 μm and greater. Total dust includes all airborne particles, regardless of their size or composition.

Excessive dust emissions can cause both health and work place problems including:

- Health hazards
  - Occupational respiratory diseases
  - Irritation to eyes, ears, nose and throat
  - Irritation to skin
- Risk of dust explosions and fire
- Damage to equipment
- Impaired visibility and accidents
- Unpleasant odors
- Problems in community relations
- Regulatory citations and fines


Healthy Lung Contaminated Lung

Excessive or long-term exposure to harmful respirable dusts may result in a respiratory disease called pneumoconiosis. Pneumoconiosis is a general name for a number of dust-related lung diseases including:

- **Silicosis** - Silicosis is a form of pneumoconiosis caused by the dust of quartz and other silicates. The condition of the lungs is marked by nodular fibrosis (scarring of the lung tissue), resulting in shortness of breath. Silicosis is an irreversible disease; advanced stages are progressive even if the individual is removed from the exposure.
- **Black Lung** - Black lung is a form of pneumoconiosis in which respirable coal dust particles accumulate in the lungs and darken the tissue. This disease is progressive. Although this disease is commonly known as black lung, its official name is coal worker's pneumoconiosis (CWP).
- **Asbestosis** - Asbestosis is a form of pneumoconiosis caused by asbestos fibers. This disease is also irreversible.

Chemical Dust Suppression Systems

Chemical Dust suppression systems can be used to reduce dust emissions. Although installing a dust control system does not assure total prevention of dust emissions, a well-designed dust control system can further protect workers and often provide other benefits such as:

- Reducing cleanup and maintenance costs
- Reducing equipment wear, especially for components such as bearings and pulleys on which fine dust can cause a "grinding" effect and increase wear or abrasion rates
- Increasing worker morale and productivity
- Assure continuous compliance with existing health and environmental regulations
- Increase plant availability and reliability
- Reduce plant water use relative to water only systems

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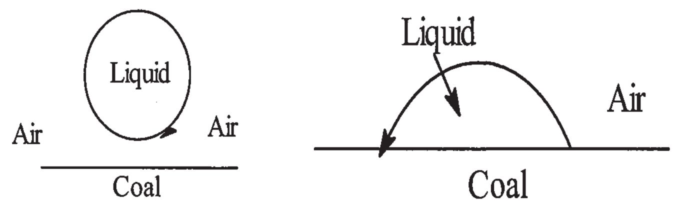
Proper planning, design, installation, operation, and maintenance are essential for an efficient, cost-effective, and reliable chemical dust suppression system. Applications for chemical dust suppression systems in the coal yard include:

- Coal transfer points
- Coal pile and car top residual, sealers & encrusting agents
- Haul road dust control
- Flow enhancers
- Washdown systems
- Yard spray systems

Application Issues

Surface Tension

Coal dust suppression is a complex phenomena; necessitating the use of surface active agents. Coal is water hating (hydrophobic), repelling water from the coal surface. In order to make the coal surface less hydrophobic, a surface active material is added to the water. The surface active material lowers the surface tension of the water to a value closer to that of the coal allowing it to be adsorbed on the surface of the coal. The water, by adsorbing on the coal surface, renders it less hydrophobic. The following figure depicts the phenomena.



Untreated coal on the left and wet coal on the right.

The surface active agent forms a microscopic liquid film as a means of increasing the adhesion of the coal dust particles. An effective dust suppressant must wet (making it less water hating) the surface of the coal, maintaining a moist environment, and bind the coal dust particles to the coal to prevent regeneration of the dust. Benetech offers a full range of optimized dust suppression products based on our expertise in surface science. These non-flammable, non-toxic, non-explosive and biodegradable products can be used to suppress dust via wetting, foaming, residual, or emulsification processes.

By evaluating contact angles and spreading coefficients of numerous surfactant molecules, Benetech has identified the structure/ property relationships of commercial surfactants and their interaction, contributing to optimum wetting and adhesion. All Benetech formulations contain synergistic combinations of wetting agents, necessary for providing fast, efficient, and effective dust suppression as well as agents to enhance foam quality and overcome problems associated with hard or brackish waters.

The figure below depicts the adsorption and adhesion phenomena associated with effective dust suppression. The wetting of the coal involves the displacement of air from

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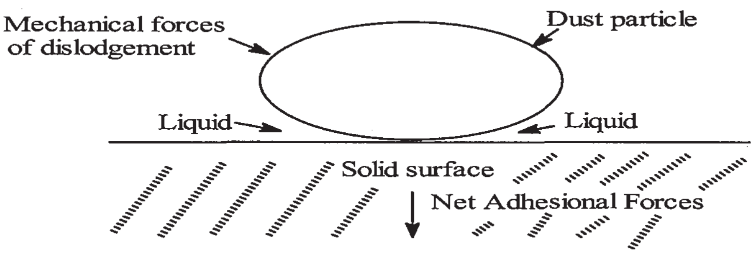
the surface by a liquid; namely, water or an aqueous solution. The addition of a surfactant to the water, by reducing the surface tension of water and perhaps the interfacial tension between the water and the coal particle making spontaneous spreading possible. This occurs in three steps involving adhesion (solid liquid interface at the expense of both liquid - gas and solid - gas) spreading (formation of liquid – gas and liquid - solid at the expense of solid – gas) and immersion (solid – gas interface is replaced by a solid – liquid) wetting.



Adsorption and adhesion phenomena associated with effective dust suppression.

Effective wetting of the coal dust can be achieved by-

- **Static Spreading** - The material is wetted while stationary. Important factors include the diameter and contact angle of water droplets. In general, surface coverage can be increased by reducing either the contact angle or droplet diameter.
- **Dynamic Spreading** - The material is wetted while moving. The droplet impact velocity, surface tension of the liquid, the material size, and the droplet diameter are important variables in dynamic spreading. The surface coverage can be Increasing the surface coverage can be achieved either by reducing the surface tension or by increasing the impact velocity.

Both static and dynamic spreading of a droplet can be increased by reducing the surface tension and thus decreasing the droplet diameter. However, the impact velocity of smaller droplets decreases faster due to frictional drag and less momentum, which, in turn, reduces dynamic spreading. An optimum droplet diameter for maximum material surface coverage must therefore be determined.

Factors Affecting Surface Wetting

Droplet Size

Surface wetting can be increased by reducing the droplet diameter and increasing the number of droplets. This can be achieved by reducing the surface tension/contact angle. The surface tension of pure water is 72.6 dyne/cm. It can be reduced from 72.6 to 28 dyne/cm by adding minute quantities of surfactants. This reduction in surface tension (or contact angle) results in-

- Reduced droplet diameter
- An increase in the number of droplets

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- A decrease in the contact angle

Impact Velocity

Surface wetting can be increased by increasing the impact velocity. Impact velocity can be increased by increasing the system's operating pressure. Due to the frictional drag of the turbulent air, the impact velocity of the droplet is less than its discharge velocity from the nozzle. Smaller droplets lose velocity faster than larger ones. To cover the greatest surface area, the best impact velocity for a given droplet diameter must be determined for each operation.

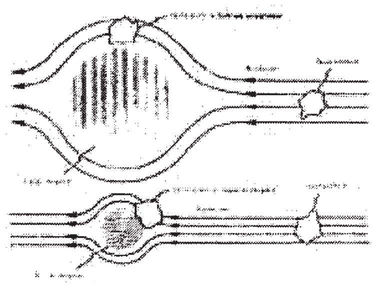
Factors Affecting Collision

The collision between dust particles and water droplets occurs due to the following three factors:

- Impaction/interception
- Droplet size/particle size
- Electrostatic forces

Impaction/Interception

When a dust particle approaches a water droplet, the airflow may sweep the particle around the droplet or, depending on its size, trajectory, and velocity; the dust particle may strike the droplet directly, or barely graze the droplet, forming an aggregate.



Effect of Droplet Size
Schowengerdt and Brown

Droplet Size/Particle Size

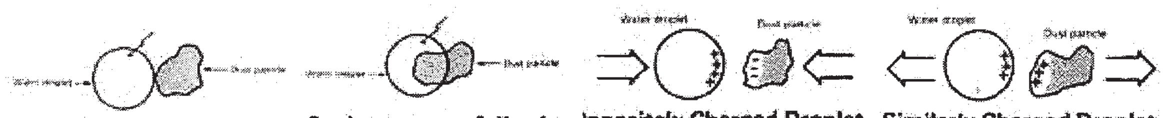
Droplets and particles that are similar in size have the best chance of colliding. Droplets or dust particles that are smaller in size relative to the particle or droplet being impacted may never collide but just be swept around one another.

Electrostatic Forces

The presence of an electrical charge on a droplet affects the path of a particle around the droplet. When particles have an opposite or neutral charge, collision efficiency is increased.

Airborne Dust Capture

When fine droplets are sprayed into the airborne dust, the droplets and dust particles collide and agglomerates are formed. When these agglomerates become too heavy to remain airborne, they settle. Wetting the bulk material also lowers the tendency to generate dust. Keeping the material damp immobilizes the dust, and very little material becomes airborne.



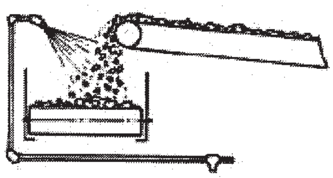
Collision Between Dust Particle and Water Droplet

Coalescence or Adhesion Between Dust Particle and Water Droplet

Oppositely Charged Droplet and Particle Attract Each Other

Similarly Charged Droplet and Particle Oppose Each Other

Finely atomized water sprays are normally used at transfer points without excessive turbulence or when the velocity of dust dispersion is less than 200 ft/min. The optimum droplet size, water usage, relative velocity, and number and location of nozzles depend on the conditions at individual transfer points.



Wet Dust Suppression System

Types of Dust Suppression Systems

Chemical Dust Suppression Systems fall into four broad categories:

- **Water Sprays with Surfactant** - This method uses surfactants to lower the surface tension of water. The droplets spread further and penetrate deeper into the coal. Surfactants can also be used to reduce the friction factor between wet coal particles and transfer surfaces to mitigate pluggage issues.
- **Foam** - Water and a special blend of surfactant make the foam. The foam increases the surface area per unit volume, which increases wetting efficiency.
- **Water Sprays with Binders, Humectants, and Surfactants** – This method uses a binder to create a longer residual suppression effect. The purpose of the humectant is to retard the moisture evaporation process. The surfactant enhances wetting.
- **Emulsions –** Emulsions of water and surfactants are used to suspend normally immiscible binders to create a residual effect suppressant. Oil and latex based emulsions are examples of suppression agents used as car top and pile sealers and road haul suppressants.

Suppression Chemicals

Based on its' expertise in surface science, Benetech offers a wide range of optimized dust suppression products in all of the above categories. These products are non-flammable, non-toxic, non-explosive and biodegradable and can be used to suppress dust via either foam or wetting action.

Coal dust suppression is a complex phenomena; necessitating the use of surface active agents. Coal is water hating (hydrophobic), repelling water from the coal surface. In order to make the coal surface less hydrophobic, a surface active material is added to the water. The surface active material lowers the surface tension of the water to a value closer to that of the coal allowing it to be adsorbed on the surface of the coal. The water by adsorbing on the coal surface renders it less hydrophobic.

The surface active agent forms a microscopic liquid film as a means of increasing the adhesion of the coal dust particles. An effective dust suppressant must wet (making it less water hating) the surface of the coal, maintaining a moist environment, and bind the coal dust particles to the coal to prevent regeneration of the dust. By evaluating contact angles and spreading coefficients of numerous surfactant molecules, Benetech has identified the structure/ property relationships of commercial surfactants and their interaction, contributing to optimum wetting and adhesion. All Benetech formulations contain synergistic combinations of wetting agents, necessary for providing fast, efficient, and effective dust suppression for a variety of coal types under both hard and brackish water conditions.

Wetters

Coal, a hydrophobic substance, is difficult to be wet with hydrophilic water. In order to wet the surface of coal a surface active agent that lowers the coal air/ air water surface tension is required. A surfactant is a material that, when present at low concentration in a system, has the property of adsorbing onto the surface of the system and of altering to a marked degree the surface properties of the system. A surfactant molecule is a molecule containing two diverse groups. It is composed of a hydrophilic (water loving) head and a hydrophobic (water hating) tail. Two fundamentally dissimilar groups within a single molecule is the most fundamental characteristic of a surfactant. The surface activity is determined by the structural makeup of the two groups. Water is a highly structured substance because of the strong hydrogen bonds between hydrogen and oxygen. When added to water the hydrophobic tail is incompatible and is rejected by the water and is ejected to the surface or interface where it forms a monolayer, thereby lowering the surface tension (See Figure). If the molecule did not contain the water loving head, it would be completely ejected and form a separate immiscible phase. Upon saturation of the solid/ air interface the surfactant forms micelles in the water solution. The concentration of the surfactant at which micelles begin to form is known as the critical micelle concentration (CMC).

The most common hydrophobe is a hydrocarbon, specifically an eight to eighteen carbon chain. The hydrophile can be anionic, cationic or nonionic. Surfactants in which the hydrophilic moiety is a sulfate or sulfonate are anionic. When the hydrophilic group is a polyether group the surfactant is nonionic.

Wet dust suppression requires the formation of microscopic liquid films as a means of increasing the adhesion of coal dust particles through hydrogen bonding. The wetting agent, because of the large surface area must adsorb on the coal particles spontaneously and efficiently. This requires the surfactant to be a highly branched and symmetrical molecule such that it diffuses rapidly from the hydrophilic water environment to the interface where it is adsorbed at the coal/air interface. The highly branched hydrophobe makes micelle formation difficult, hence increasing the number of monomers in solution making diffusion to the interface more rapid and promoting better wetting.

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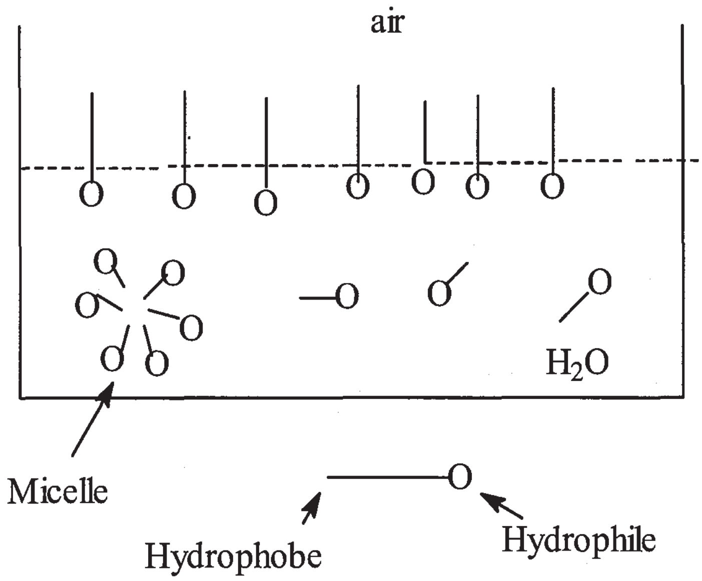
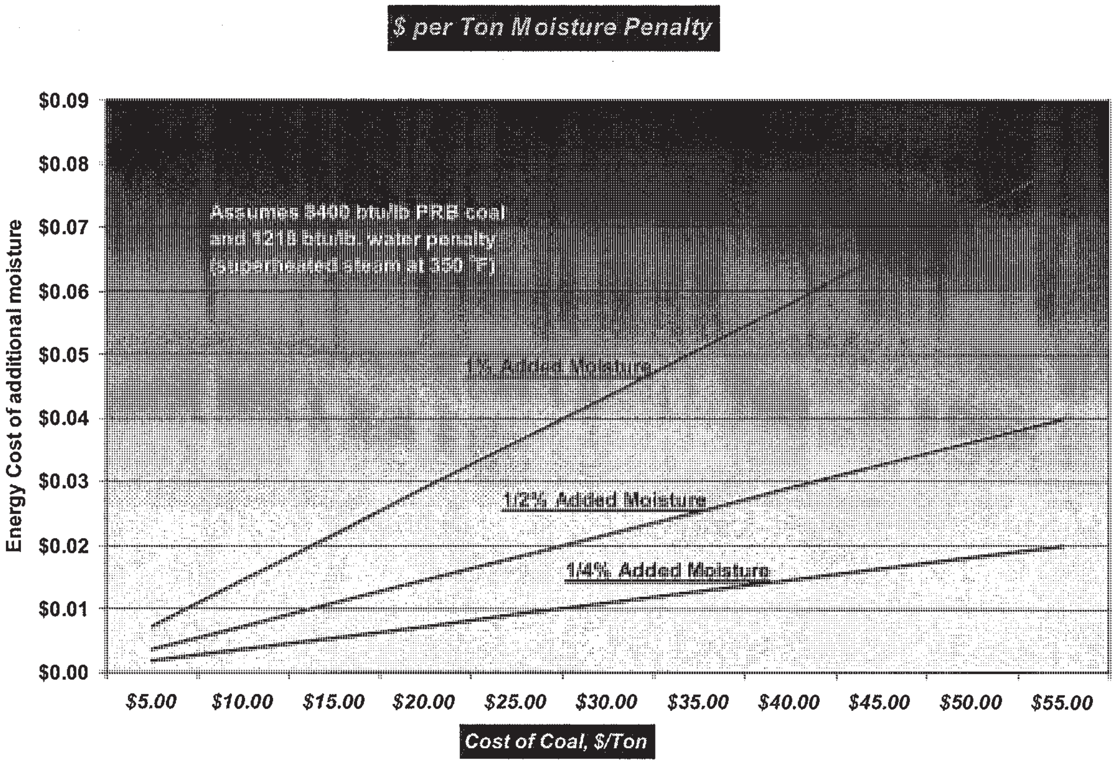


Foamers

Aqueous foamable compositions can be used to suppress coal dust particles. A unique property of foam is its ability to blanket a mass of coal, thereby forming a physical barrier against the dispersion of dust. The foam barrier makes it difficult for the coal dust particles to get airborne. It is preferable to trap the particles before they get airborne. Foam suppression is useful in situations where the quantity of available water is limited or it is desirable to limit the amount of water applied to the coal. The penalty for excess water addition is presented in the following Figure.

Foaming agent formulations frequently contain a wetting agent and a polymer to give body to the foam and reduce the chances of the coal particles becoming airborne after the foam has collapsed. The foam generated is preferably a small bubble foam (100 to 200 microns) allowing efficient trapping of dust particles. A stream of foam interacts with coal particles especially the larger fines. When the foam bubbles impact the coal dust particles, the particles are wetted by the imploding bubbles and captured. Many fine droplets are also released from the collapsing foam, which scrub more fine coal particles. The same principles governing the interaction of coal dust particles with surfactant solutions also govern their action with foam systems.

Foam is a non-equilibrium dispersion of gas bubbles in a relatively smaller volume of liquid. Pure liquids do not foam. Foam is produced when a gas is introduced into a solution whose surface film has viscoelastic properties. The resulting foam possesses a honeycomb arrangement. An essential ingredient in liquid based foam is surface active molecules. These materials reside at the air/ liquid interface and are responsible for both the tendency of a liquid to foam and the stability of the resulting dispersion of gas bubbles. Just as surfactants self-organize (form micelles) in the bulk solution as a result of their hydrophilic and hydrophobic segments, they also preferentially adsorb and organize at the solution – vapor interface. In the case of the aqueous surfactant solution, the tails protrude into the vapor and leave only the hydrophilic heads in contact with the solution. The favorable energetics of the arrangement can be observed and measured by the reduction in the interfacial surface tension. The surfactant concentration is at or slightly above the CMC in most optimized foam situations. At concentrations below the CMC the liquid/ air surface is not saturated and the foam effectiveness is reduced. At concentrations considerably above the CMC the solution loses its film elasticity and the bubbles will collapse. While, the reduced surface tension is not in itself responsible for the foaming; the primary benefit is that less mechanical energy need be supplied to create the large interfacial area in foam.



Many factors promote foam formation. Low equilibrium surface tension, the smaller the cross sectional area the molecule occupies at the air/liquid interface the lower the surface tension and the closer packed the film. A high bulk phase viscosity promotes a slow draining rate for the bubbles and hence more foam. A moderate surface phase viscosity, a moderate rate of attaining equilibrium surface tension and presence of electrical double layer in the surface film also contribute to increased foam. The design of an efficient foam dust suppressant formulation requires a delicate balance of foam wetting properties, as well as consideration of water hardness.

Residual Suppressants

In order to maintain the suppression of the dust for long periods of time, a polymeric hydrophilic material is added. The polymer whether it is anionic or nonionic forms mixed micelles and mixed monolayers with the primary wetting agent. The mixed monolayers are then adsorbed onto the coal. The high molecular weight polymeric material effectively forms a shield preventing the escape of the moisture and ensures that dust particles remain stuck or adhered to each other and to the bulk coal. The polymer also acts as a nucleating agent allowing the micelles to more efficiently form and holding them in the area of the coal. Due to the large molecular weight of the polymers and the presence of hydrophilic groups, they can bind with several coal particles increasing the effective density of the coal particles preventing dusting. Typical polymers include compounds such as ligninsulfonate and polyacrylamides. It is thought that polyacrylamides may serve to reduce the rate of evaporation of water and thereby extend the life of the treatment. In addition to ligninsulfonates, a wide range of other binding agents has been used for long term coal dust control. These major classes of binders include:

- Polymer solutions
- Polymer emulsions
- Oils and oil emulsions
- Asphalt and asphalt emulsions

Humectants

Residual dust suppressant systems must also maintain the moisture content to prevent regeneration of dust. Consequently, the Benetech formulation contains both a humectant and a binder. The humectant is a water loving material which forms strong hydrogen bonds with water making its' removal from the system difficult. When humectants are used alone, such as salts (commonly used for haul road dust control), they have to be used in large amounts. Commonly employed salts include calcium, magnesium, and sodium chlorides and their mixtures. Surfactants are frequently combined with hygroscopic salts to improve the extent of coal dust capture and binding.

Emulsions

Polymer Emulsions

The largest single application of polymer emulsions is for pile sealing and railcar top coating prior to shipment to prevent dust formation and coal loss from the car tops. Latex emulsions, similar to those used in the paint industry are typically deployed. Surfactants are usually added to improve its coal wetting ability.

Asphalt and Asphalt Emulsions

Asphalt or asphalt emulsions have also been used in coal dust control. The use of an asphalt emulsion, in combination with surfactants to wet the coal rapidly, has also been used for rail car top coating and stackout pile sealing. An interesting aspect of these emulsions is their ready ability to "break". Certain surfactant solutions can be used to pre-wet the coal, so that a subsequently applied asphalt emulsion will break to leave a dust suppressing film on the coal surface.

Oils and Oil Emulsions

The use of oil as a coal surface treatment has a long history. Not only does a thin oil film provide an antidusting effect, it also adds heating value to the coal and improves the coal's bulk density – a factor of importance in coke making. Oil emulsions have the advantage that they can be diluted with water for better dispersion. Oil-soluble surfactants can also improve the antidusting properties of oils for treating coal. Oil emulsions can also "break" when they come into contact with certain surfactants.

Laboratory Testing

The effectiveness of a surfactant in modifying the wetting properties of a liquid can be evaluated by determining the spreading coefficient of the surfactant solution. This can be done by measuring both the surface tension of the surfactant solution, and the contact angle the solution makes with the substrate. The Walker and "Drop Box" test represent other common methods for evaluating surfactant effectiveness. The Walker test, first proposed by Walker and co-workers in 1952 was the first laboratory procedure to measure coal dust wetting. In this procedure, approximately 1 gram (1/4 teaspoon) of <200 mesh coal is gently floated on the surface of an aqueous solution of water plus the wetting agent. The time it takes for the coal dust to completely sink is measured and reported. Pure water shows wetting times measured in hours, where even small concentrations of some wetting agents will give wetting times of less than five minutes. This test is useful for evaluating in the laboratory the effectiveness of a given wetting or residual formulation. There is a strong inverse correlation between wetting time and initial dust suppression.

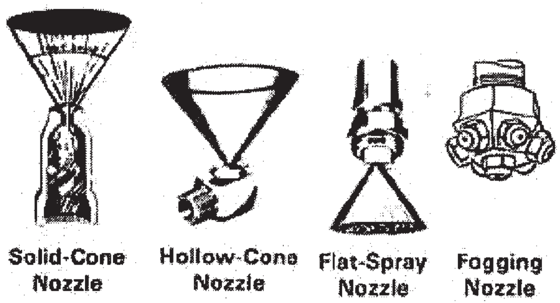
Surfactants are also known to interact with each other producing synergistic enhancement of wetting effects. Wetting agents, especially anionic and nonionic surfactants exhibit synergistic behavior, promoting a more rapid diffusion to the wetting front. Preferred surfactant systems have an optimized ratio of several surfactants. By using mixtures of surfactants one can use less surfactant than would be required for a single surfactant system.

Design of a Water-Spray System

Dust particles need to be trapped in the air and before they become airborne. An important factor in trapping air borne dust particles is the droplet size of the sprayed formulation. Droplets with a clean surface have higher capture efficiencies for dust particles than droplets already containing a trapped particle. The best surfactant system will rapidly remove the trapped dust particle to the interior of the droplet. Consequently, a coarse droplet will more efficiently capture dust than a smaller droplet. The droplet size must also be optimized with the surfactant wetting system for effective suppression. It is easiest and most desirable to knockdown the coal dust particles before they become airborne. This is accomplished by the wet dust suppression formulations.

The spray nozzle is the heart of a water-spray system. Therefore, the physical characteristics of the spray are critical. Factors such as droplet size distribution and velocity, spray pattern and angle, and water flow rate and pressure all vary depending on the nozzle selected. Following is a general discussion of these important factors:

- **Droplet Size**- The nozzle's droplet size distribution is the most important variable for proper dust control. The droplet size decreases as the operating pressure increases. Information about the droplet size data at various operating pressures can be obtained from the nozzle manufacturer. For wet dust suppression systems, coarse droplets (200-500 µm) are recommended. For airborne dust capture systems, very fine droplets (10-150 µm) may be required. The fine droplets usually are generated by fogging nozzles, which may use either compressed air or high-pressure water to atomize water in the desired droplet range.
- **Droplet Velocity** - Normally, higher droplet velocities are desirable for both types of dust control through water sprays. Information on the droplet velocity can be obtained from the nozzle manufacturer.
- **Spray Pattern** - Nozzles are categorized by the spray patterns they produce:
  - Solid-cone nozzles product droplets that maintain a high velocity over a distance. They are useful for providing a high-velocity spray when the nozzle is located distant from the area where dust control is desired.
  - Hollow-cone nozzles produce a spray patter in the form of circular ring. Droplet range is normally smaller than the other types of nozzles. They are useful for operations where dust is widely dispersed.
  - Flat-spray nozzles produce relatively large droplets that are delivered at a high pressure. These nozzles are normally useful for wet dust suppression systems.
  - Fogging nozzles produce a very fine mist (a droplet size distribution ranging from submicron to micron). They are useful for airborne dust control systems.



- **Spray Angle** - Each nozzle has a jet spray angle. The size of this angle is normally available from the manufacturer. A knowledge of spray angle and spray pattern is essential to determine the area of coverage and, therefore, the total number of nozzles needed.
- **Flow Rate** - The flow rate of water through a nozzle depends on the operating pressure. The flow rate and operating pressure are related as follows:

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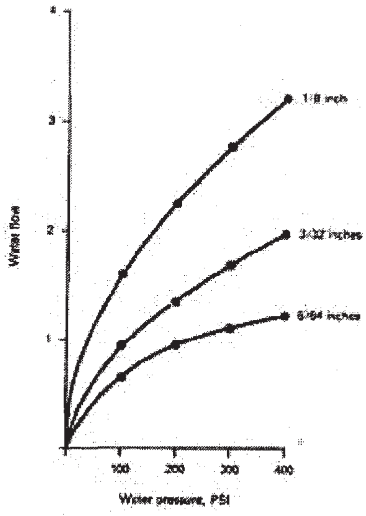
$$\text{Water flow rate} = K\sqrt[2]{\text{operating pressure}}$$

where K = nozzle constant



Water Flow/Pressure

Knowledge of the water flow rate through the nozzle is necessary to determine the percentage of moisture added to the material stream. The following factors should be considered in selecting the nozzle location:

- It should be readily accessible for maintenance.
- It should not be in the path of flying material.
- For wet dust suppression systems, nozzles should be **upstream** of the transfer point where dust emissions are being created. Care should be taken to locate nozzles for best mixing of material and water. For airborne dust capture, nozzles should be located to provide **maximum time** for the water droplets to interact with the airborne dust.

Water Flow and Compressed Airflow Rates

Once the nozzle is selected, its spray pattern and area of coverage can be used to determine water flow rate and/or compressed airflow rates and pressure requirements. These must be carefully coordinated with the maximum allowable water usage. Water flow rates will be highly variable depending on the size and type of coal, the application location, and the throughput of coal.

Piping Design

The piping should be designed so that each nozzle receives water or compressed air at specified flow rates and pressures. Drains must be provided at the lowest point in each sub circuit of the piping system to flush the air and water lines in winter months. Heat trace and insulation must also be provided at locations where the temperature may drop below 32° F. The heat tracing should be able to provide approximately 5 watts per linear foot for water pipes up to 2 in. in diameter. The pump and other hardware, such as valves and gauges, should also be placed in a heated enclosure or heat traced and insulated to prevent freezing during winter months.

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Instruments

Pressure and flow gauges are recommended to monitor system performance. These instruments should be located as close to the point of application as possible. For situations where it is desirable to activate wet suppression systems only when the material is flowing (for example, if the belt conveyor is running empty, water sprays need not be on), a solenoid-activated valve may be installed in the water line. The solenoid can be activated by instruments such as the level controller or zero speed switch. This approach will reduce water usage, reduce maintenance and cleanup, and reduce or prevent freeze up problems. It is important that electrical, control, and instrumentation meet local condition electrical code requirements. This is typically NEMA 4/9 and Class 2, Div 1, Groups F for dust.

Application Locations

Chemical dust suppressants can be used to control dust at a number of locations. These include:

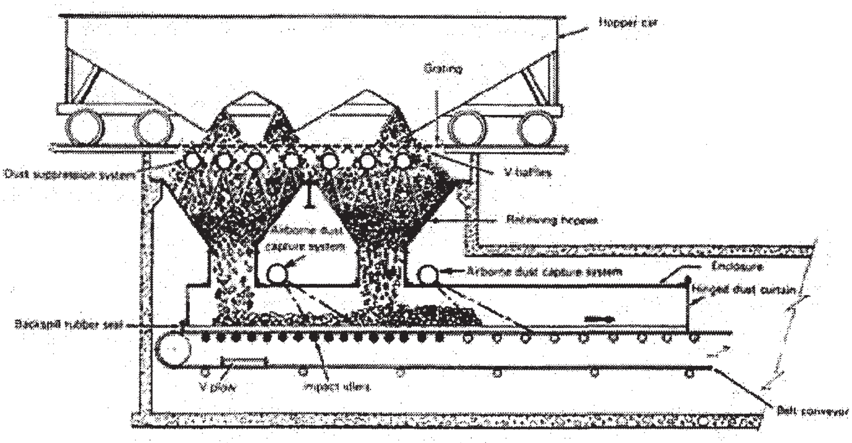
- Coal Transfer Points
- Coal Pile, Car Top, Residual, Sealers and Encrusting Agents
- Haul Road Dust Control
- Flow Enhancers
- Washdown Systems
- Yard Spray Systems
- Flyash Pug Mills

Selection of the application points will be based on a number of factors such as degree of needed dust control, need for downstream dust control, desire for residual control especially on piles and haul roads, restriction on water use, proper spray access to material, availability of support utilities (i.e., water, air, electricity), ease of support equipment placement (i.e., sheds, chemical storage tanks), and length of required supply piping.

In the coal yard, typical application locations include:

- Rail unloading system hopper area
- Barge unloading system hopper area
- Trestle rail unloading area
- Truck / payloader unloading hopper area
- Hopper reclaim feeders/transfer points
- Transfer chutes within towers
- Prior to stackout for stackout dust control as residual dust control from piles
- Bucket reclaim area on stacker / reclaimers
- Yard spray systems to provide residual effectiveness
- Sizing and crushing areas
- Washdown systems to enhance cleaning and reduce water consumption
- In chute areas to reduce wet coal pluggage
- At trippers, cascade, and reversing decks to control dust emissions within the plant and in bunkers and silos

The following diagram presents a typical chemical dust suppression arrangement at a bottom dump rail unloading system. As with all applications, site specific conditions will influence exact spray nozzle header locations to maximize contact with airborne dust and the bulk coal material. Good chemical dust suppression systems can reduce dust levels over 90%, reduce respirable dust below OSHA requirements of 2.0 μm/m^3, and maintain opacity levels below 10%.



Railroad Dump to Conveyor

PNAS PLUS

Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 *Cosco Busan* oil spill in San Francisco Bay

John P. Incardona[a,1], Carol A. Vines[b], Bernadita F. Anulacion[a], David H. Baldwin[a], Heather L. Day[a], Barbara L. French[a], Jana S. Labenia[a], Tiffany L. Linbo[a], Mark S. Myers[a], O. Paul Olson[a], Catherine A. Sloan[a], Sean Sol[a], Frederick J. Griffin[b], Karl Menard[b], Steven G. Morgan[b], James E. West[c], Tracy K. Collier[a,2], Gina M. Ylitalo[a], Gary N. Cherr[b,d], and Nathaniel L. Scholz[a]

[a]Environmental Conservation Division, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, WA 98112; [b]University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923; [c]Marine Resources Division, Washington State Department of Fish and Wildlife, Olympia, WA 98501; and [d]Departments of Environmental Toxicology and Nutrition, University of California, Davis, CA 95616

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ENVIRONMENTAL SCIENCES

In November 2007, the container ship *Cosco Busan* released 54,000 gallons of bunker fuel oil into San Francisco Bay. The accident oiled shoreline near spawning habitats for the largest population of Pacific herring on the west coast of the continental United States. We assessed the health and viability of herring embryos from oiled and unoiled locations that were either deposited by natural spawning or incubated in subtidal cages. Three months after the spill, caged embryos at oiled sites showed sublethal cardiac toxicity, as expected from exposure to oil-derived polycyclic aromatic compounds (PACs). By contrast, embryos from the adjacent and shallower intertidal zone showed unexpectedly high rates of tissue necrosis and lethality unrelated to cardiotoxicity. No toxicity was observed in embryos from unoiled sites. Patterns of PACs at oiled sites were consistent with oil exposure against a background of urban sources, although tissue concentrations were lower than expected to cause lethality. Embryos sampled 2 y later from oiled sites showed modest sublethal cardiotoxicity but no elevated necrosis or mortality. Bunker oil contains the chemically uncharacterized remains of crude oil refinement, and one or more of these unidentified chemicals likely interacted with natural sunlight in the intertidal zone to kill herring embryos. This reveals an important discrepancy between the resolving power of current forensic analytical chemistry and biological responses of keystone ecological species in oiled habitats. Nevertheless, we successfully delineated the biological impacts of an oil spill in an urbanized coastal estuary with an overlapping backdrop of atmospheric, vessel, and land-based sources of PAC pollution.

embryology | heart development | free radical | forensic chemistry | natural resource injury assessment

On November 7, 2007, the container ship *Cosco Busan* collided with the San Francisco–Oakland Bay Bridge, breaching two port fuel tanks and spilling approximately 54,000 gallons of bunker fuel into San Francisco Bay. This spill occurred in the spawning and rearing habitat for the largest coastal population of Pacific herring (*Clupea pallasii*) along the continental United States (1). Herring are a keystone species in the pelagic food web, and this population supports the last commercial finfish fishery in San Francisco Bay.

The *Cosco Busan* spill visibly oiled shorelines adjacent to North Central Bay areas where herring have historically spawned (November through March; Fig. 1). Although visibly oiled shorelines were cleaned, some extensively, only 52% of the oil was recovered from surface waters and land or lost to evaporation (2). The amount of hidden or subsurface oil that may have remained near herring spawning areas is unknown. The toxicity of crude oil to herring early life stages was extensively documented in the aftermath of the 1989 *Exxon Valdez* spill, which also occurred contemporaneously to Pacific herring spawning in Prince William Sound, Alaska (3–6). Therefore, the contamination of intertidal and shallow subtidal zones with *Cosco Busan* bunker oil posed a toxic threat to herring spawn and by extension, the productivity and abundance of the San Francisco Bay spawning population. Adult herring deposit adhesive, demersal eggs on shallow nearshore vegetation and other substrates. This proximity to oiled shorelines increases the risk of herring embryo exposure to water-soluble, toxic compounds that derive from oil as it weathers (i.e., shifts in chemical composition) over time.

Research on Alaska North Slope crude oil following the *Exxon Valdez* spill, together with studies during the past two decades that used other sources of crude oil and other teleost species, have yielded two central insights (7). First, teleost embryos exposed to trace concentrations of crude oil constituents dissolved in water exhibit a common syndrome of developmental abnormalities (8–17). Gross features include pericardial and yolk sac edema, small jaw, and body axis defects. Of these, edema appears to be the most sensitive indicator of oil exposure and toxicity (9, 11, 18). Second, studies in zebrafish and Pacific herring have shown that petroleum-induced edema is cardiogenic. Specifically, crude oil contains a directly cardiotoxic fraction attributable to the most abundant polycyclic aromatic compounds (PACs), the tricyclic fluorenes, dibenzothiophenes, and phenanthrenes (17–20). These compounds interfere with the physiological function of the embryonic heart, producing cardiac arrhythmia in herring embryos at tissue concentrations as low as 0.8 μmol/kg wet weight (19). Although embryos with edema do not survive as feeding larvae, externally normal embryos surviving Alaska North Slope crude oil exposure grew up to be adults with subtle changes in heart shape and reduced aerobic (i.e., swimming) capacity (21).

Based on this previous work, we initiated a 3-y study beginning 3 mo after the *Cosco Busan* spill to assess PAC exposure, sublethal cardiac toxicity, developmental abnormalities, and hatching success in herring embryos in San Francisco Bay. We moored cages

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[1]To whom correspondence should be addressed. E-mail: john.incardona@noaa.gov.

[2]Current address: Oceans and Human Health Initiative, National Oceanic and Atmospheric Administration, Silver Spring, MD 20910.

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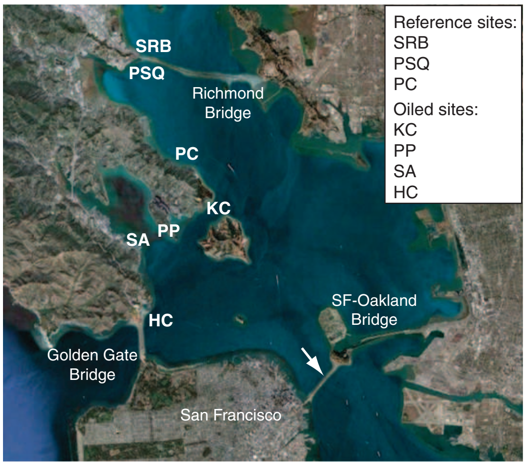

Fig. 1. Satellite view of study sites in central San Francisco Bay. Landmarks include the Golden Gate Bridge to the west and the Richmond Bridge to the north. Oiled sites include HC, SA, PP, and KC. Nonoiled reference sites include PC, PSQ, and SRB. Arrow indicates location of oil release.

containing artificially fertilized embryos, together with passive water sampling devices for PACs (i.e., polyethylene membrane devices; PEMDs), at six sites. Four of these sites were visibly oiled immediately after the spill, whereas two "reference" sites were not oiled but contiguous with the same heavily urbanized shoreline (Table 1 and Fig. 1). We also collected naturally spawned embryos from five intertidal sites, four of which were adjacent to the caged embryos (Table 1 and Fig. 1). At the time of embryo incubations, there was no visible oiling at any of the sites, although small tar balls were occasionally found on shore. Embryos from all sites were transported to a laboratory for live imaging by using digital photo- and videomicroscopy and for incubation to hatching. We measured PACs and a suite of persistent organic pollutants (POPs) routinely found in urban environments, including polychlorinated biphenyls (PCBs) and organochlorine pesticides, in embryos in 2008 and 2010. Additionally, ovaries and whole bodies of prespawning adult herring entering San Francisco Bay in 2008 were analyzed for PACs and POPs to evaluate the potential for maternal transfer of contaminants.

Results

Cardiac Defects in Caged Embryos Incubated in Subtidal Zone at Oiled Sites. Sublethal cardiac effects were observed in caged embryos placed subtidally at oiled sites in 2008 (Fig. 2). Pronounced bradycardia was observed in embryos from 13 of 17 cages deployed at the four oiled sites, with significant variability among cages at a given site (Fig. 2 *A* and *B*). At three of the oiled sites, embryos from all cages measured showed a significant bradycardia in the range of 90 beats/min [Horseshoe Cove (HC), 90 ± 1; Sausalito (SA), 92 ± 1; and Peninsula Point (PP), 92 ± 1]. Only one of five cages at the Keil Cove (KC) oiled site showed significant bradycardia (cage 1, 95 ± 1 beats/min), and the overall mean (114 ± 2 beats/min) was statistically indistinguishable from that in reference sites. There was no significant variation among heart rates within the five cages from each of the two reference sites (Fig. 2*A*), and both sites had an overall mean heart rate of 116 ± 1 beats/min (Fig. 2*B*). Mean temperatures were indistinguishable among sites (*SI Appendix*, Table S1), indicating that differences in heart rate were not caused by incubation temperatures. Hatching success of caged embryos was high, with average normal hatch rates (i.e. percentage of hatched larvae with normal morphology) ranging from 79 ± 5% to 97 ± 2% at all sites, compared with 91 ± 1% in controls held back in the laboratory (*SI Appendix*, Fig. S2). With all types of morphological abnormalities pooled, all oiled sites, but also reference site Point San Quentin (PSQ), had hatch rates (normal larvae) significantly lower than those in reference site San Rafael Bay (SRB) and laboratory controls. However, pericardial edema, which is produced by more severe impairment of cardiac rhythm in developing fish embryos, was observed only at each oiled site in a small but significant percentage of larvae hatched from subtidal cages, ranging from 0.9% to 2.5% (Fig. 2*C*). In contrast, no edema was observed among 652 larvae hatched from subtidal cages at either reference site (PSQ, $n = 308$; SRB, $n = 344$).

Necrotic Late Embryos in Natural Spawn Samples from Intertidal Zone at Oiled Sites. Abundant natural spawn occurred on macroalgae in the lower intertidal zone at four of the six sites where caged embryos were deployed in adjacent subtidal zones in 2008, comprising three oiled and one reference site (Table 1 and *SI Appendix*, Table S2 and Fig. S3). High rates of lethality and morphological abnormalities occurred in natural spawn from all three oiled sites. At the hatching stage, healthy herring embryos are normally translucent and colorless except for a darkly pigmented eye and a row of individual melanophores ventrally along the gut. This normal condition was observed in all embryos from each subtransect from reference site SRB (Fig. 3*A*). Upon dechorionation, SRB embryos uncurled (Fig. 3*B*) and were motile. Despite having developed fully pigmented eyes and ventral melanophores (indicating normal development approaching the hatching stage), the majority of embryos in samples from oiled sites were dead on examination in the laboratory, as indicated by

Table 1. Physical shoreline characteristics of sample sites and types of samples collected each year

Site	SCAT rating	Cleanup	Adjacent land use/maritime use	Subtidal sampling: Incubated caged embryos*	Subtidal sampling: PEMDs	Intertidal sampling*
KC	Oiled; heavy	Extensive wiping, removal of rock	Residential, undeveloped forest	2008	Yes	2008, 2010
HC	Oiled; moderate-light	Extensive wiping of rip-rap	Marina, major highway	2008	Yes	ND
SA	Oiled; very light-light	Some wiping	Marina, commercial, residential	2008	Yes	2008, 2010
PP	Oiled; light	Some wiping	Residential	2008	Yes	2008, 2010
SRB	No oil	NA	Commercial parking lot, major highway	2008	Yes	2008
PC	No oil	NA	Residential, public green space	Not sampled	No	2009, 2010
PSQ	No oil	NA	Commercial/industrial parking lots, major highway	2008	Yes	Not sampled

NA, not applicable; ND, no spawn detected; SCAT = shoreline cleanup assessment team.
*All caged and naturally spawned embryos were assessed for sublethal exposure to PACs and POPs, except the 2009 samples.

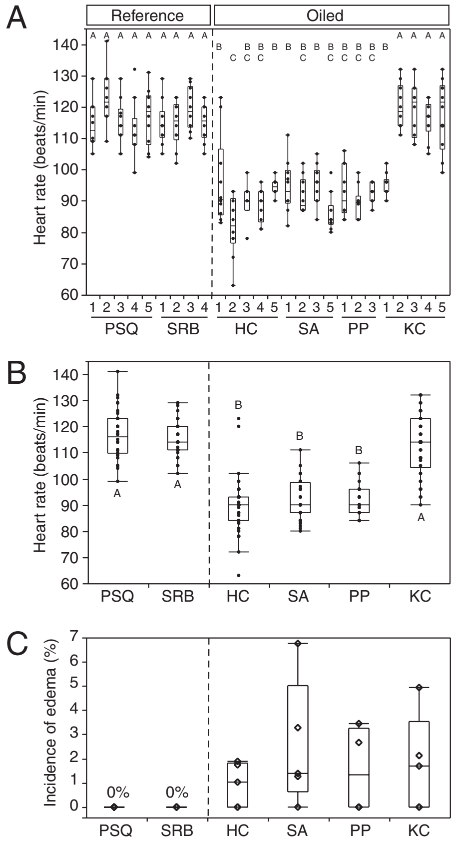

Fig. 2. Evidence of cardiac dysfunction in embryos and larvae incubated in subtidal cages at oiled sites in 2008. Box-and-whisker plots encompass the distribution of individual data points. (*A*) Mean heart rate ($n = 30$) by cage. One cage at SRB was lost at retrieval, one cage at PP was stripped of embryos before retrieval, and video from one cage each at SA and PP were of insufficient quality to provide heart rate data. (*B*) Mean heart rate by site. Mean heart rates from individual cages were pooled for a site mean. ANOVA indicated 34% of the total variance was caused by the cage, and showed significant effects of site ($P < 0.001$) and oiled state ($P < 0.001$). Letters A–C indicate statistically similar datasets identified by post-hoc means comparison with Tukey-Kramer HSD test ($\alpha = 0.05$). Overall, oiled sites were statistically different from reference sites (Student *t* test, $\alpha = 0.05$). (*C*) Incidence of pericardial edema in larvae hatched in the laboratory after incubation to 7 d postfertilization in subtidal cages. Edema tended to be observed more frequently at oiled sites (Wilcoxon rank-sum test, $P = 0.06$).

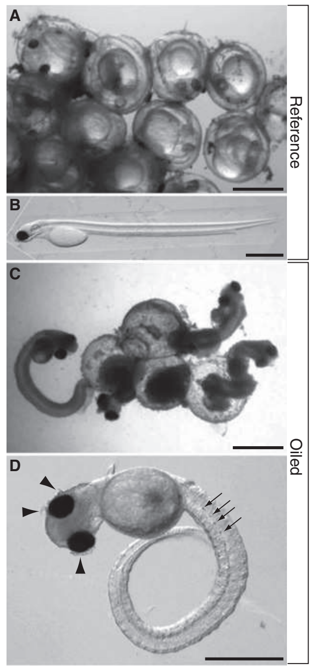

Fig. 3. Representative images of intact and dechorionated naturally spawned embryos from oiled and nonoiled intertidal sites sampled in 2008. Images were made by using oblique coherent contrast illumination on a Nikon SMZ1000 stereomicroscope, allowing high relief for colorless, transparent specimens. (*A*) An undissected cluster of embryos in the chorions from reference site SRB with normal translucency. Eyes are darkly pigmented and the trunk and tail are coiled around the brightly transparent yolk. (*B*) Representative embryo from SRB uncoiled after dechorionation, with anterior to the left and dorsal at top. Longitudinal features evident with oblique coherent contrast illumination from dorsal to ventral are the transparent neural tube, notochord, and gut. (*C*) An undissected cluster of nonviable embryos from oiled site PP showing pigmented eyes, opacity indicative of deteriorating tissues, and spontaneously ruptured chorions. (*D*) Representative late-stage embryo from PP with cardiac activity after dechorionation. Arrows indicate ventral pigment cells, arrowheads indicate loss of epidermal integrity.

absence of heart beats and motor activity (Movie S1), opacity of tissues such as the brain, spinal cord, and axial muscle, and disintegration of epidermal tissue (Fig. 3*C*). Because it was impossible to gain etiological insight from these necrotic embryos, we focused our image analysis on a small subset of living embryos that exhibited obviously beating hearts. Most of the living embryos from oiled sites exhibited opaque tissues, failure to straighten the body axis, and immotility (Fig. 3*D* and Table 2). In many cases, there was also loss of epidermal integrity (Fig. 3*D*, arrowheads). We consistently observed cardiac dysfunction at all three oiled sites in 2008 (i.e., arrhythmia, silent ventricle, severe bradycardia, minimal overall contractility, and loss of heart beat; Table 2), defects that were entirely absent in embryos from SRB.

In 2008, essentially no live larvae hatched from the natural spawn we collected from oiled sites SA and PP, and samples from oiled site KC had a normal hatch rate of only 14 ± 13% (Fig. 4). In contrast, at reference site SRB, hatching began within 1 day following collection, and 71 ± 10% of embryos hatched to produce morphologically normal larvae (Fig. 4). Herring did not spawn in 2009 at any of the sites that had been oiled in 2008, but they did spawn in 2009 at a third nonoiled reference location [Paradise Cove (PC); sampled in January]. The natural spawn from PC in 2009 occurred at a higher elevation in the intertidal zone than the naturally spawned sites sampled in 2008, and were distributed on rocks as well as macroalgae. A relatively high proportion of 2009 embryos from this site failed to gastrulate (28 ± 7%); nevertheless, viable embryos from PC produced a normal hatch rate of 77 ± 4% (Fig. 4).

In 2010, herring once again spawned at several locations where natural spawn was collected in 2008. The overlap was exact at two oiled sites (PP, KC) and partial at a third oiled site (SA). (To distinguish areas of overlap between 2008 and 2010, the SA site was subsequently divided into an inner-marina SA1 subsite and outer SA2 subsite; Table 3 and *SI Appendix*, Fig. S1*A*). Herring also spawned again at the PC reference site,

Table 2. Incidence of abnormalities in viable embryos in natural spawn collected from oiled and nonoiled sites in 2008

Site	Body axis defect	Tissue opacity	Edema	Arrhythmia
SA (oiled)	60 ± 11	41 ± 10	33 ± 7	48 ± 7
PP (oiled)	98 ± 2	96 ± 2	11 ± 4	91 ± 11
KC (oiled)	90 ± 5	86 ± 7	11 ± 2	70 ± 6
SRB (nonoiled)	0	0	1.0 ± 0.6	0

Table shows mean percent and SEM from eight samples (≥20 embryos per sample) at each site.

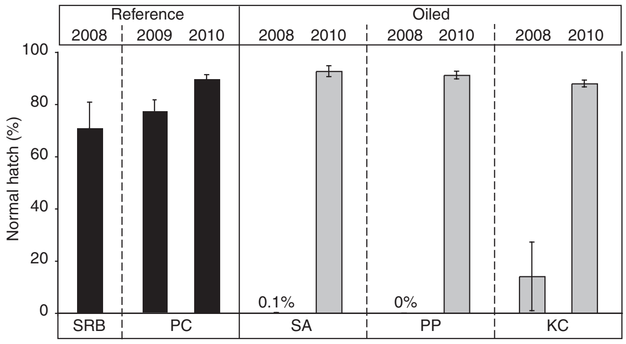

Fig. 4. Hatching rates of larvae from natural spawn samples. Values represent mean ± SEM normal hatch rates, calculated as percent morphologically normal larvae hatched from total eggs in eight transect subsamples per site. Grand totals of eggs assessed for each site were 535 (SRB, 2008), 820 (PC, 2009), 683 (PC, 2010), 968 (SA, 2008), 385 (SA, 2010), 549 (PP, 2008), 919 (PP, 2010), 469 (KC, 2008), and 726 (KC, 2010).

at an intertidal depth equivalent to the samples collected in 2008. Larvae at all three oiled sites had normal cardiac and spontaneous motor activity (Movie S1). Furthermore, hatching rates were similar among the oiled and reference sites (Fig. 4), and these were similar to the relatively high hatching rates for nonoiled reference sites sampled in previous years (SRB in 2008 and PC in 2009). However, we observed a significantly higher incidence of pericardial edema in larvae from oiled KC site (3.5 ± 0.7%) relative to the other sites [SA, 1.6 ± 0.3%; PP, 1.0 ± 0.3%; and PC, 1.2 ± 0.3%; ANOVA, $P < 0.003$, Tukey–Kramer honestly significant differences (HSD) test].

Analyses of PACs and POPs in Prespawn Adults and Embryos. Measurements of PACs and POPs in prespawn adult herring entering San Francisco Bay in February 2008 suggest that maternal transfer did not contribute significant levels of these compounds to embryos (*SI Appendix*, Table S3). Summed PACs (∑PACs) were very low (near the limit of detection), with wet weight concentrations ranging from 8.6–13 ng/g in ovaries and only one quarter the concentration (23–52 ng/g) of maternal whole bodies. Dichlorodiphenyltrichloroethanes (DDTs) and PCBs were the most abundant classes of POPs (2.8 ± 0.3 ng/g and 1.6 ± 0.4 ng/g, wet weight, respectively) detected in whole bodies; levels of DDTs were higher and PCBs lower than in prespawn adult herring from Puget Sound, Washington (22) (*SI Appendix*, Table S3). Other classes of POPs (e.g., chlordanes, polybrominated diphenyl ethers, hexachlorocyclohexanes) were also detected in whole bodies of herring but were below quantification limits in ovaries. Consistent with adult ovary data, PCB levels in natural spawn were low, near detection limits (*SI Appendix*, Table S3).

In caged and naturally spawned embryos, comparison of individual classes of PACs representing petrogenic or pyrogenic sources revealed significant differences among sites (Table 3), despite generally low mean ∑PAC concentrations that were in many cases statistically indistinguishable from maternal ovary levels according to standard parametric tests (complete data provided in Dataset S1). Sulfur-containing dibenzothiophenes are petrogenic PACs and often used for characterizing sources of oil in the environment (23–26). Although *Cosco Busan* bunker oil is low in sulfur compared with other fuels, with a C2-dibenzothiophene/C2-phenanthrene ratio of 0.26, the alkyl-dibenzothiophenes are nevertheless one of this bunker oil's most abundant identified PAC classes. Total alkyl-dibenzothiophene levels tended to be higher in natural spawn embryos from each oiled site and in caged embryos from three of four oiled sites than from nonoiled sites in 2008 (Wilcoxon rank-sum test, $P = 0.08$). C2- and C3-dibenzothiophene together were also detected more frequently in caged and naturally spawned embryos from oiled sites in 2008. In 2010, alkyl-dibenzothiophenes in natural spawn were not detected at oiled site PP but remained significantly elevated at oiled site KC (ANOVA, $P = 0.03$; Tukey–Kramer HSD, $\alpha = 0.05$). The pyrogenic PAC fluoranthene appeared to be more uniformly distributed. Slightly lower fluoranthene levels at reference sites were not statistically significant ($P = 0.28$, Wilcoxon rank-sum test), and there was only a weak correlation between mean fluoranthene and total alkyl-dibenzothiophene concentrations ($r^2 = 0.6$).

Distinct Patterns of PAC Inputs Evident from Analysis of PEMD Passive Samplers. A comparison of PAC patterns in PEMDs deployed alongside the caged embryos indicated distinct PAC chemical fingerprints among the six subtidal sites (Fig. 5) that seemed to reflect their nearby upland development. Moreover, as expected for an urbanized estuary, all sites had mixed inputs of petrogenic

Table 3. Summary of selected PAC concentrations (ng/g wet weight) measured in naturally spawned and caged embryos collected in 2008 and 2010

Matrix/y (*n*)	Site	Mean ∑PACs*	Mean FLA	Mean Alkyl-DBTs†	Frequency C2/C3-DBT, %‡
Cage/2008 (4)	SRB (reference)	17 ± 3	0.8 ± 0.3	Below LLOQ	0
Spawn/2008 (8)	SRB (reference)	21 ± 2	1.1 ± 0.2	0.05 ± 0.03	0
Cage/2008 (5)	PSQ (reference)	23 ± 2	1.0 ± 0.2	0.09 ± 0.06	0
Spawn/2010 (8)	PC (reference)	28 ± 3	0.5 ± 0.1	0.05 ± 0.05	13
Cage/2008 (4)	HC (oiled)	52 ± 10	3.7 ± 0.9	0.48 ± 0.23	50
Cage/2008 (5)	SA (oiled)	48 ± 6	2.7 ± 0.5	0.51 ± 0.13	80
Spawn/2008 (5)	SA1 (oiled)	81 ± 40	2.9 ± 0.7	0.49 ± 0.29	20
Spawn/2008 (3)	SA2 (oiled)	18 ± 3	0.6 ± 0.1	Below LLOQ	0
Spawn/2010 (3)	SA2 (oiled)	27 ± 1	1.4 ± 0.1	Below LLOQ	0
Cage/2008 (4)	PP (oiled)	21 ± 1	0.8 ± 0.1	Below LLOQ	0
Spawn/2008 (8)	PP (oiled)	19 ± 5	0.8 ± 0.1	0.12 ± 0.08	25
Spawn/2010 (8)	PP (oiled)	23 ± 1	0.6 ± 0.1	Below LLOQ	0
Cage/2008 (5)	KC (oiled)	24 ± 3	0.7 ± 0.1	0.21 ± 0.10	40
Spawn/2008 (8)	KC (oiled)	45 ± 18	3.8 ± 2.2	0.28 ± 0.09	75
Spawn/2010 (8)	KC (oiled)	34 ± 9	1.8 ± 1.0	0.48 ± 0.16	100

DBT, dibenzothiophene; FLA, fluoranthene.
*Mean and SEM values from given *n*.
†Lower limit of quantification (LLOQ) values for individual analytes ranged from < 0.15 to < 0.46; samples with values lower than the LLOQ were treated as 0 in means.
‡Frequency of samples with both C2- and C3-DBT detected.

and pyrogenic PACs (*SI Appendix*, Table S4). Nonmetric multidimensional scaling (nMDS) and an analysis of similarity (ANOSIM) revealed that PAC patterns were highly significantly different among sites ($P = 0.001$, $R = 0.69$; Fig. 5). A pairwise ANOSIM test showed that sites HC and SA, which have marinas and were oiled, exhibited similar PAC patterns ($R = 0.33$) and were both dissimilar to all other sites ($R = 0.85$–1.0). Similarly, reference sites PSQ and SRB, which are on opposite sides of a major highway (Richmond Bridge; Fig. 1), were indistinguishable from each other ($R = 0.26$) and were isolated from all other sites ($R = 0.85$–1.0). Oiled sites PP and KC, which are residential or minimally developed, were isolated from each other and all other sites ($R = 0.85$–1.0). Diagnostic PAC ratios also showed differences among sites (*SI Appendix*, Table S4). The ratio of sum alkyl-phenanthrenes to phenanthrene (MP/P) is widely used to distinguish pyrogenic from petrogenic sources (27), with the latter having MP/P ratios greater than 2. Conversely, the ratio of fluoranthene plus pyrene to sum C2- through C4-phenanthrene increases with increasing pyrogenic composition (26). The MP/P ratio was greater than 2 for only the most heavily oiled site, KC (MP/P ratio, 2.6), whereas the ratios of fluoranthene plus pyrene to C2- through C4-phenanthrene clustered into four groups that matched the nMDS analysis. This analysis is consistent with heterogeneous background PAC inputs among the six sites that are associated with the adjacent upland development and land use, as well as an elevation of petrogenic signal above the background level at the heavily oiled site KC.

Discussion

Unlike the *Exxon Valdez* oil spill that occurred in Prince William Sound, Alaska, the *Cosco Busan* spill occurred within a highly urbanized estuary with multiple inputs of petroleum hydrocarbons. This posed the difficult challenge of assessing the ecological impacts of the spill against the background of pollution in San Francisco Bay. Based on years of oil toxicity research since the *Exxon Valdez* spill, we anticipated that lingering oil toxicity, if any, would be evident as a small increase in the detection of sublethal cardiac effects (e.g., arrhythmia, edema) in herring embryos incubated near oiled shorelines. Although significant increases in bradycardia and pericardial edema were observed in caged embryos from oiled sites relative to nonoiled locations, natural spawn from oiled intertidal zones revealed an unexpectedly severe (i.e., lethal) form of developmental toxicity.

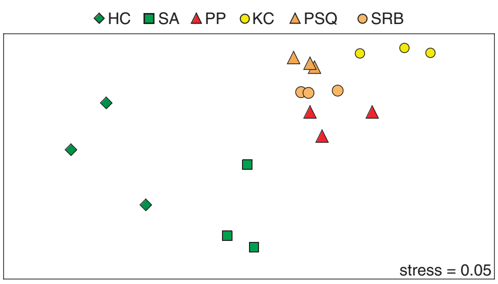

Fig. 5. Comparison of PAC patterns in PEMDs deployed at six sites indicates distinct PAC sources. nMDS was used to represent a large number of PAC compounds in low-dimensional (i.e., 2D) space. nMDS analysis was carried out using Primer version 6.0 as described in *SI Appendix*. Axes surround a unitless space within which samples are placed according to the degree of similarity in the relative abundance of five influential PAC compounds (preselected from 34 compounds using the Primer BEST routine). Similarity in PAC patterns determines the distance between points in the space: samples with similar patterns are placed close together and dissimilar patterns are further apart. The observed patterns are statistically different from a random configuration of points (stress, 0.05). PSQ and SRB are reference sites; HC, SA, PP, and KC are oiled sites.

The high rate of natural spawn mortality at oiled sites in 2008 does not appear attributable to natural causes or anthropogenic causes unrelated to the spill. The spawning layers were not dense enough (i.e., fewer than four layers at all sites) to cause the density-dependent hypoxia that occurs when eggs are deposited in layers of greater than eight eggs thick (28–33). Euryhaline Pacific herring embryos develop normally at salinities of 8% to 28% (34, 35), and even suboptimal salinities would not be expected to cause acute mortality late in development. Natural spawn did not show evidence of accelerated development, as would be expected following exposures to high, potentially lethal temperatures (34, 36). Coating of herring eggs with fine sediments does not produce the late developmental mortality we observed here (37–41). Two sewage spills occurred during the 2008 spawning season; on January 13 in Richardson Bay, near oiled sites SA and PP, and on February 14 offshore of San Quentin Prison, near reference site PSQ. However, the available evidence indicates that sewage (i.e., concentrated sludge) is not acutely lethal to herring embryos (42). Finally, background PCB and DDT levels in ovaries and embryos from San Francisco Bay are not expected to cause the observed mortalities, as the levels were much lower than that associated with reduced hatching success in Baltic herring (43).

Our chemical analyses of PACs in embryos and PEMDs support the conclusion that embryos from oiled sites were exposed to oil, particularly at KC, even though a PAC "fingerprint" of *Cosco Busan* oil was not discernable against the background of urban PAC inputs. In particular, the trends of higher levels and increased frequency of alkyl-dibenzothiophene detections in embryos at oiled sites are consistent with lingering oil exposure at those sites that were visibly oiled several months before spawning. The PEMD data indicated unique patterns of PAC inputs across sites, consistent with the diversity of proximal land use patterns and vessel activities. However, embryonic phenotype did not follow this pattern of site-specific chemical variation. In contrast, the effects observed in natural spawn from each of the three oiled sites (SA, PP, and KC) were indistinguishable, and pericardial edema was observed only in larvae that were incubated subtidally at oiled sites. The only common feature linking these sites was a shoreline presence of visible oil in the weeks following the spill. The absence of these effects at reference sites and the marked recovery at oiled sites by 2010 indicate that background urban inputs of contaminants (e.g., via stormwater) were not likely causal. The most parsimonious explanation for the 2008 herring embryo mortality in San Francisco Bay is exposure to *Cosco Busan* oil.

PAC levels in the tissues of embryos collected from oiled intertidal sites in 2008 were below levels that would be expected to cause acute lethality based on laboratory studies (11, 19, 44). This, together with the dramatic difference in survival between intertidal spawn and embryos in nearby subtidal cages, implicates natural sunlight as a contributing factor in the observed embryolarval toxicity. Sequential exposures to crude oil and sunlight in the laboratory are acutely lethal to herring larvae at tissue PAC concentrations that are only sublethal without sunlight exposure (45). This presumably occurs via activation of PACs or other oil components by UV radiation (i.e., "phototoxicity"), thereby generating reactive oxygen species that cause membrane damage (46). Other studies in aquatic invertebrates and fish demonstrated that some bunker oils, as well as other petroleum sources that are low in conventionally measured PACs, have higher phototoxicity than predicted from the measured PACs (47, 48). Recent work in zebrafish has further shown that bunker oils have a much higher phototoxic potential than crude oil and cause an acutely lethal cellular necrosis when embryos are exposed sequentially to oil and sunlight (49). Finally, a 2009 laboratory study to investigate the potential role of sunlight in the observed 2008 natural spawn mortality showed that *Cosco Busan* bunker oil exhibited a phototoxic activity that (*i*) produced late embryonic mortality in herring embryos characterized by a loss of tissue integrity similar to that observed in the 2008 field-collected samples, (*ii*) was resistant to weathering, and (*iii*) was unexplained by conventionally measured tissue PAC concentrations, thus suggesting a causal role for one or more unmeasured compounds (50). Because modern bunker fuels contain the concentrated residuum of the crude oil refining process, they have much higher relative levels of many compounds, including the uncharacterized polar compounds that make up an "unresolved complex mixture" (51). The simplest explanation for our collective findings is that an uncharacterized and slowly weathering component of *Cosco Busan* bunker oil accumulated in the naturally spawned herring embryos, and then interacted with sunlight during low tides to produce lethal phototoxicity. Embryos in nearby cages, submerged beneath approximately 1 m or more of highly turbid San Francisco Bay water, exhibited canonical oil toxicity (i.e., bradycardia and pericardial edema) with no indication of a sunlight interaction.

Research following the *Exxon Valdez* oil spill established a new paradigm for oil toxicity to fish at early life stages (7), which demonstrated the central role PACs play in causing characteristic developmental deformities. Our ecological assessments of the *Cosco Busan* spill have extended and reinforced this paradigm by (*i*) highlighting the difference in effects on fish from exposure to oil of differing composition (i.e., crude vs. bunker), (*ii*) illustrating the potential significance of photo-enhanced toxicity and its interaction with local conditions, (*iii*) providing motivation to examine toxicity of a broader suite of petroleum product components, and (*iv*) emphasizing the exceptional vulnerability of fish early life stages to spilled oil. Our research showed strong evidence for a previously undescribed lethal toxicity in Pacific herring embryos associated with exposure to bunker oil, which may have been exacerbated by exposure to sunlight. Although bunker oil spills are typically smaller in volume than crude oil spills, they may have disproportionate impacts in ecologically sensitive, sunlit habitats.

Materials and Methods

Study Sites. Sites were selected based on their likely proximity to oiled substrates as determined by Shoreline Cleanup Assessment Team surveys and other observations of oiling. Because the sites (Fig. 1 and Table 1) differed in the degree of oiling and cleanup, the actual amounts of residual oiling (including subsurface oil) at each location at the time of the assessment are unknown. Reference, nonoiled sites were chosen to be representative of an urban estuary and most closely match the habitat, temperature, and salinity conditions at corresponding sites within the spill zone. Satellite images indicating natural spawn subtransects and caged embryo/PEMD placements are shown in *SI Appendix*, Fig. S1.

General descriptions of natural spawn sample substrates are provided in *SI Appendix*, Table S2. The predominant substrates were brown and red bladed algae such as *Fucus*, *Cryptopleura*, and *Chondracanthus*, filamentous red algae (e.g., *Gracilaria, Microcladia*, or *Odonthalia*), and some green algae (*Ulva*). The highest-density spawn was at reference site SRB, where samples were almost exclusively on *Fucus*. *Fucus* also predominated at SA, but macroalgae were more variably mixed at PP and KC, with the former predominated by filamentous red forms. Typical samples are shown in *SI Appendix*, Fig. S2. The spawn at SRB was medium in density, approaching four layers of embryos. Spawning at the three oiled sites was less dense, ranging from very light "salt-and-pepper" density to light density with contiguous patches of a single layer.

Collection of Tissues from Adult Herring for Analysis of Maternal Contaminant Exposure. Ovaries and whole bodies were collected from prespawning adult females to determine levels of POPs and PACs in ovaries and whole bodies to evaluate potential maternal transfer. Ovaries from the same 10 fish were placed into individual precleaned 240-mL glass jars (I-Chem), and the carcasses placed in individual zip-lock bags after recording fork length and weight. All tissue samples were frozen until analyzed. Bile was processed and analyzed for FACs, and ovaries and whole bodies for PACs and POPs, as described in the subsequent sections.

Collection of Herring Gametes and Preparation of Embryos. Ripe prespawn herring were captured by hook-and-line, cast net, or midwater trawl, immediately sexed, and transferred to clean zip-lock bags and stored in a clean cooler on ice. Herring gonads were dissected and eggs fertilized as described elsewhere (35). Test fertilizations were conducted with eggs from individual females, and those with high fertilization success (≥90%) were pooled for mass fertilizations. Three separate catches of fish were used among the sites. Mean (± SEM) female weights for sites were as follows: HC and PP, 82.2 ± 13.6 g (n = 7); SA, KC, and SRB, 70.3 ± 8.4 g (n = 12); and PSQ, 78.7 ± 4.1 g (n = 20). Milt was pooled from five males for each experiment. Gametes were stored at 4 °C for a maximum of 72 h to allow a successive series of daily cage deployments. Eggs were kept from clumping before fertilization with polyvinyl alcohol (35), and were distributed onto 13 × 54-cm nylon mesh sheets for mounting in cages (15-cm^3 lidded aluminum baskets; Fisher Scientific). The top and bottom of each cage was lined with the same nylon mesh, and each sheet with adhering fertilized eggs was then folded to line the sides of the cages, with the corners secured in place with cable ties. Eggs faced the interior of the cage, which was effectively lined entirely with nylon mesh to prevent the entrance of small egg-eating predators. Before transport for deployment, cages were placed in large zip-lock bags containing seawater diluted to 16 psu with Millipore-filtered water and held in clean 20-L plastic buckets. Cages were transported to sites on ice in coolers, and deployed as follows: HC, February 10, 2008; PP, February 11; SA, February 12; KC, February 13; SRB, February 14; and PSQ, February 18.

Deployment of Embryo Cages, PEMDs, and Data Loggers. Anchor-buoy units for embryo cages and PEMDs consisted of a braided polypropylene line attached to a pair of concrete blocks joined by cable ties for a combined weight of 27 kg. Each line had two floats, a 20-cm inflatable surface marker, and a smaller crab-pot buoy mounted at a position to float on the surface at low tide. The latter served to maintain a vertical line no matter the tide level. Cages and PEMDs were attached with cable ties below the smaller float at a point 18 to 30 cm above the concrete blocks. Anchor-buoy units were installed along an isobath at −0.9 to −1.7 m, 1 to 2 d before embryo cage deployment, to allow any disturbed bottom sediments to clear.

The vessel used for cage and PEMD deployment was powered with a four-stroke engine. The vessel was fueled in advance of all operations and was cleaned after fueling and before operations to avoid contamination. Mooring locations were approached downwind, and all work related to deployment/retrieval of cages/PEMDs was from the bow or center. A designated boat operator handled all mechanical systems on board, and separate personnel carried out deployments. Upon arrival at a site, the bagged cages were lowered to a free diver in the water, followed by subsurface removal of the zip-lock bag and attachment to the mooring line by using two heavy-duty cable ties interlaced into the strands of the mooring line. PEMDs were handled similarly, with removal of aluminum foil wrap below the surface. When these had been secured, global positioning system coordinates and exact depth of water were documented. After a 7-d period of incubation at the field sites, retrieval of cages and PEMDs essentially reversed this process, with bagging below the surface before being handed onto the vessel. Sealed bags with cages were placed in a cooler for transfer to the laboratory.

Salinity and temperature data were collected from one anchor-buoy unit per site by using a SeaStar DST CTD recorder (Star-Oddi) attached to an embryo cage in a mesh bag. The recorders were activated for testing with salinities and temperature confirmed by using a refractometer and handheld thermometer 12 to 24 h before deployment, and programmed to collect temperature and conductivity data at 30-min intervals. Data were downloaded from recorders in Microsoft Excel format.

Field Collection of Naturally Spawned Herring Eggs. Locations of naturally deposited herring spawn were determined by a combination of vessel-based vegetation rake sampling, direct observation by free diving, or direct observation of intertidal spawn from shore at low tide. The turbidity of San Francisco Bay hinders direct visual observation of deposited subtidal spawn by divers. Although major deposits of subtidal spawn were not detected in the vicinity of the cage moorings at any of the study sites in 2008, intertidal spawning was estimated to have occurred on or close to February 19, 2008, at the SRB reference site, spreading westward through the range of the study area through February 22. Natural spawn samples were collected at the reference site SRB (February 22), and oiled sites SA (February 27), KC (February 28), and PP (February 29). Although natural spawning was observed at the PSQ reference site, the spawning density was very light, occurred concurrently with a sewage spill from the adjacent San Quentin prison, and was concurrent with much more dense spawn deposition at SRB. As a result of personnel exposure considerations and logistics of processing samples, it was decided to not collect samples at PSQ. Intertidal spawning was observed in only one location in 2009: PC, a reference site not sampled in 2008. Spawning occurred beginning the week of January 19, 2009, at PC, and spawn was sampled on January 28. In 2010, intertidal spawning occurred at some study sites starting January 26.

Spawn samples were collected from oiled sites PP (February 4), SA (February 4), KC (February 5), and reference site PC (February 6).

At all sites, samples were collected 7 d after the natural spawning event had originally been detected by rake or shore-based surveys, or based on field examination of the embryo developmental stage at each site by visual inspection after fixation in Stockard solution. At each location, attempts were made to collect marine vegetation with spawned herring eggs attached from the middle to lower intertidal zone. These efforts were successful in most situations. At some sites, marine vegetation was largely absent in the lower intertidal zone, and the herring had primarily spawned in the upper intertidal zone. In these situations, samples were collected as low in the intertidal zone as possible, and in no cases were samples collected above the waterline.

The protocol for collection of natural spawn was performed uniformly at all sampling sites. At each site (Fig. S1), samples were collected along a 100-m transect at positions shoreward of the cage deployment positions (i.e., C1–C5) and parallel to the shore within the intertidal zone. Each 100-m transect was divided into 10 distinct 10-m subtransects from which vegetation samples with attached spawn were pooled into eight distinct samples (i.e., N1–N8); two subtransects within the 100-m transect were randomly skipped at each site. GPS coordinates were recorded at the midpoint of each subtransect. Samples were collected from the shoreline by personnel wearing chest waders and/or by free diving. Algal holdfasts were cut with a knife, and the entire sample placed in heavy-duty zip-lock bags containing ambient seawater. When a sufficient sample size for processing the required laboratory subsamples had been collected at each subtransect, the zip-lock bag was filled with ambient water at the same subtransect, sealed, and placed in a large cooler lined with freshly frozen blue ice. Individual samples from the same site were separated from one another by frozen blue ice blocks, to maintain an ambient, or lower than ambient, water temperature during transport back to the laboratory. In all cases, processing of the natural spawn samples in the laboratory was begun within 6 h after the last natural spawn subtransect site was collected in the field.

Laboratory Processing of Embryos for Analytical Chemistry, Imaging, and Hatching Assay. Upon arrival, general condition of samples were assessed. Nylon sheets with caged embryos were removed, cut into thirds, and placed in 150-mm plastic Petri dishes filled with 16 psu seawater on ice. From one subsample, 2 to 3 g of embryos for PAC analysis were scraped with an inverted 100-mm Petri dish into a precleaned 240-mL glass jar (I-Chem) and frozen at −20 °C. The other subsamples were used for dechorionation and assessment of live embryos and incubation to hatch. Embryos were dechorionated with no. 55 Dumont forceps and anesthetized with a dose of MS-222 titrated just to achieve immobilization. At least 30 undamaged embryos were collected from each cage (150 total per site), and imaged in 16 psu seawater in a 100-mm Petri dish containing 1% agarose molded with slots to accommodate the eye and yolk sack for lateral views. Embryos were examined on Nikon SMZ-1000 stereoscopes maintained at 12 °C with temperature-controlled stages (Brook Industries). Digital imaging was performed with Fire-i400 digital video cameras, with still images acquired on Apple PowerBook G4 laptops using BTV Carbon Pro-5.4.1, and 20-s video clips of the heart were collected with iMovie HD (Apple). Processing of natural spawn samples was similar, with vegetation divided into the three subsamples (collection of 100 embryos for freezing intact in liquid nitrogen, 2–3-g composite samples for analytical chemistry, and imaging and hatching). Embryos were removed from vegetation with forceps individually or in clumps. For each of the eight transects in 2008 and 2009 samples, at least 20 embryos were imaged, for a total of 160 per site. In 2010, natural spawn samples were assessed more broadly while still attached to vegetation. Three subsamples of algae with attached eggs were taken from each of the eight transects, and scanned microscopically for the presence of necrotic or grossly abnormal embryos. Images of eight random fields were collected for each subsample, with each image containing at least 10 embryos (total of ≥240 embryos per site). Because essentially no gross defects or abnormal mortality were observed in 2010 samples, embryos were not dechorionated for detailed morphological analysis, but morphology was assessed in hatched larvae.

For hatching rates, a section of mesh from cages containing as many as 200 embryos (assessed macroscopically) was placed into 250-mL glass culture dishes containing 200 mL 16 psu seawater and incubated in a 12 °C incubator. Most of the sites showed evidence of hatching (i.e., empty chorions observed microscopically) before retrieval, so initial actual numbers of embryos were lower. Daily water changes were performed for the duration of incubation and counts were performed daily for 2 to 6 d, based on the variability in days to hatching observed between sites. For natural spawn samples, several strands of vegetation with attached embryos were placed into 11 × 21-mm rectangular glass dishes containing 600 to 700 mL 16 psu seawater and incubated in a 12 °C incubator. As many as 100 embryos were carefully removed from the vegetation into six-well culture plates (20–30 embryos per well) and incubated at 12 °C with daily water changes. Forty-eight hours after retrieval, embryos, larvae, and empty chorions (i.e., egg shells) were enumerated as follows: eyed nonhatched embryos, dead or unfertilized embryos, number of empty chorions, and normal and abnormal larvae. Subsequent daily counts enumerated only eyed nonhatched embryos, partially hatched embryos, and abnormal larvae. As a result of adherence of sediments or other suspended particles to caged embryos, it was difficult to determine incidence of nonfertilized vs. embryos with arrested development; thus, these embryos were counted as dead/unfertilized. Partially hatched larvae (i.e., embryos/larvae that had partially exited the chorion but were nonviable) were counted as nonhatched embryos. Larvae were defined as normal if they had straight body axes, lack of pericardial or yolk sac edema, regularly beating hearts, and ability to swim and respond to stimuli (i.e., touch). Normal hatching for both cages and natural spawn was defined as the number of morphologically normal larvae per total number of hatched and unhatched embryos combined.

Analyses of PACs and POPs in Herring Tissues, and PEMDs. Herring tissues (embryos, adult bodies, and ovaries) were extracted and analyzed for PACs and POPs by using a GC/MS method described elsewhere (52, 53). Briefly, this method involves (*i*) extraction of tissues using an accelerated solvent extraction procedure, (*ii*) clean-up of the entire methylene chloride extract on a single stacked silica gel/alumina column, (*iii*) separation of PACs and POPs from the bulk lipid and other biogenic material by high-performance size-exclusion liquid chromatography, and (*iv*) analysis on a low-resolution quadrupole GC/MS system equipped with a 60-m DB-5 GC capillary column. The instrument was calibrated by using sets of as many as six multilevel calibration standards of known concentrations. PEMDs were extracted after applying an internal standard solution to each sample, with a spiking solution of analytes added to a subset. The samples were extracted in 20%/80% methylene chloride/pentane by sonication, and extracts eluted through silica columns by using 20%/80% methylene chloride/pentane to remove any interfering biogenic compounds. The cleaned-up extracts were concentrated to 100 μL and analyzed as described for eggs and sediments.

Statistics. Statistical analyses were carried out by using JMP 6.0.2 (SAS Institute) for Macintosh (Apple). Heart rate data were analyzed by a mixed-model nested ANOVA, with cages nested under site as a random effect, site nested under oiled state, and oiled state as an independent variable. PAC levels were compared by one-way ANOVA. In cases in which ANOVA results were significant, differences among sites were compared post hoc by using the Tukey–Kramer HSD test, which compares all means to each other. The probabilities of a nonrandom distribution for the incidence of edema and levels of alkyl-dibenzothiophenes and fluoranthene were determined by using the nonparametric Wilcoxon rank-sum test with the site mean values. For analyses of PAC concentration data, values that were below the limits of quantification were treated as 0 in calculations of means.

Analyses of PAC Patterns in PEMDs. The relative abundance of 34 PAC compounds in PEMDs was compared between sites by using multidimensional scaling analysis (MDS) (54) in the software package Primer version 6 (55). In brief, the MDS algorithm attempts to satisfy conditions prescribed by a PAC-similarity matrix to place samples with similar PAC patterns together, and dissimilar samples apart in low-dimensional (i.e., 2D) space with the least amount of stress. PAC data were pretreated by standardizing (i.e., computing the proportional contribution of each PAC compound concentration to the total PAC concentration in each sample), and then transforming by taking square root, to reduce the contribution of dominant compounds. The total number of PACs input to the MDS procedure (34) was reduced to five compounds that most efficiently described the PAC patterns, by using the BEST procedure (Primer version 6) to eliminate compounds that did not contribute to explaining the observed PAC patterns. Subsequent Bray–Curtis similarity data for the five compounds selected by the BEST procedure were plotted in 2D, on unitless axes. Pairwise site comparisons of PEMD patterns were conducted with ANOSIM, using the R statistic to identify the main between-site differences. Values of the ANOSIM R statistic range from 0 (i.e., no separation, or complete similarity) to 1.0 (i.e., complete separation, or no similarity) of sites.

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